Technical Manual

GenePrint® Fluorescent STR Systems

(For use with the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems, ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 and 3100 Genetic Analyzers.)

INSTRUCTIONS FOR USE OF PRODUCTS DC5171, DC6070, DC6071, DC6171, DC6300, DC6301, DC6310, DC6311, DG2121, DG2131 AND DG3291.

www.promega.com

PRINTED IN USA
Revised 8/12

Part# TMD006

GenePrint® Fluorescent STR Systems

All technical literature is available on the Internet at: www.promega.com/protocols/
Please visit the web site to verify that you are using the most current version of this Technical Manual.
Please contact Promega Technical Services if you have questions on use of this system.
E-mail: genetic@promega.com

1. Description 2

2. Product Components and Storage Conditions 4
- A. *GenePrint*® Fluorescent STR Multiplex Systems 4
- B. Allelic Ladders and Size Markers 5
- C. *GenePrint*® Fluorescent Sex Identification Systems 5
- D. *GenePrint*® Fluorescent Monoplex Systems 5

3. Before You Begin 6
- A. Precautions 6
- B. Matrix Standardization or Spectral Calibration 6

4. Amplification 7
- A. Choice of Thermal Cycling Protocol 8
- B. Amplification Setup 12
- C. Amplification Thermal Cycling 14

5. Polyacrylamide Gel Preparation 15
- A. Gel Preparation for the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems 15
- B. Gel Preparation for the ABI PRISM® 377 DNA Sequencer 18

6. Polyacrylamide Gel Electrophoresis and Detection 19

7. Sample Preparation, Gel Electrophoresis and Detection on the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems 20
- A. Gel Pre-Run 20
- B. Sample Preparation, Loading and Electrophoresis 21
- C. Detection 22
- D. Data Analysis 22
- E. Reuse of Glass Plates 23

8. Sample Preparation, Gel Electrophoresis and Detection on the ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 Genetic Analyzer 24
- A. Instrument Preparation 24
- B. Sample Preparation and Loading 25
- C. Electrophoresis and Detection 26
- D. Data Analysis 27
- E. Reuse of Glass Plates 27

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA · Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**

Printed in USA.
Revised 8/12

Part# TMD006

9. **Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer and Data Collection Software, Version 1.1** 28
A. Sample Preparation....28
B. Instrument Preparation....29
C. Sample Detection....30
D. Data Analysis....31

10. **Representative STR Data**....31

11. **Troubleshooting**....34

12. **References**....36
A. Cited References....36
B. Additional STR References....38

13. **Appendix**....38
A. Advantages of STR Typing....38
B. Advantages of Using the Loci in the *GenePrint*® Fluorescent STR Systems....39
C. Power of Discrimination....42
D. DNA Extraction and Quantitation Methods....43
E. Agarose Gel Electrophoresis of Amplification Products....44
F. Composition of Buffers and Solutions....45
G. Related Products....46

1. Description

STR[(a)] (short tandem repeat) loci consist of short, repetitive sequence elements of 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using PCR (5–8). Alleles of these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using radioactive, silver stain or fluorescence detection following electrophoretic separation.

The *GenePrint*® Fluorescent STR Systems contain all materials, except for *Taq* DNA polymerase and sample DNA, required to perform 100 or 400 amplification reactions. Accessory components are available to simplify many of the procedures related to STR analysis (Section 13.G).

All *GenePrint*® Fluorescent STR Systems contain 10X primer pairs. In each pair, one primer is labeled with fluorescein (FL), and the matched primer is unlabeled. In the *GenePrint*® Fluorescent Identification System—TMR, the Amelogenin-specific primer is labeled with tetramethylrhodamine (TMR). STR 10X Buffer, loading solution, the appropriate allelic ladder and K562 DNA (positive control template) are also provided.

The *GenePrint*® Fluorescent STR Systems can be detected using any of the following instruments: Hitachi FMBIO® and Hitachi FMBIO® II Fluorescence Imaging Systems, ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 and 3100 Genetic Analyzers.

This manual describes methods that we have evaluated and recommend to prepare samples, amplify samples, separate amplified products and detect separated material. Instructions to operate fluorescence-detecting instrumentation should be obtained from the instrument manufacturer.

The *GenePrint*® Fluorescent STR Multiplex Systems CSF1PO, TPOX, TH01, vWA (CTTv); F13A01, FESFPS, F13B and LPL (FFFL); and D16S539, D7S820, D13S317 and D5S818 (GammaSTR®) are currently quality certified on the Hitachi FMBIO® II Fluorescence Imaging System. The CTTv Multiplex System and *GenePrint*® Fluorescent Sex Identification System—Amelogenin are quality certified for amplification using the PerkinElmer Model 480 thermal cycler, while the GammaSTR® Multiplex System is quality certified for amplification using the PerkinElmer GeneAmp® PCR System 9600 thermal cycler. The FFFL Multiplex is optimized for use on the GeneAmp® PCR System 9700 thermal cycler.

All *GenePrint*® Fluorescent Systems can be amplified on the PerkinElmer Model 480 or GeneAmp® PCR System 9600 or 9700 System thermal cycler, but slight differences in yield or balance between loci might be observed if the system was not optimized on that particular thermal cycler. This manual provides a number of cycling protocol options so that excellent results can be obtained regardless of the thermal cycler used.

Allele frequencies for African-Americans, Caucasian-Americans and Hispanic-Americans for all currently available STR systems can be found at: **www.promega.com/techserv/apps/hmnid/referenceinformation/popstat/custstat_Allelefreq.htm**. Additional population data for STR loci can be found in references 3 and 9–13. Additional STR references are listed in Section 12.B.

2. Product Components and Storage Conditions

2.A. *GenePrint*® Fluorescent STR Multiplex Systems

Below is a description of the components of the *GenePrint*® Fluorescent STR Multiplex Systems. All *GenePrint*® STR Multiplex Systems include the required fluorescein-labeled 10X primer pairs as a mixture for simultaneous amplification of more than one locus and a mixture of the fluorescein-labeled allelic ladders for the same set of loci. Additional components include STR 10X Buffer, K562 DNA, loading solutions and Gel Tracking Dye.

Product	Size	Cat.#
GenePrint® Fluorescent STR Multiplex— CSF1PO, TPOX, TH01, vWA (Fluorescein)[a,b]	100 reactions	DC6301
	400 reactions	DC6300
GenePrint® Fluorescent STR Multiplex— GammaSTR® (Fluorescein)[a,c] D16S539, D7S820, D13S317, D5S818	100 reactions	DC6071
	400 reactions	DC6070
GenePrint® Fluorescent STR Multiplex— F13A01, FESFPS, F13B, LPL (Fluorescein)[a,d]	100 reactions	DC6311
	400 reactions	DC6310

Not for Medical Diagnostic Use. Cat.# DC6300, DC6070 and DC6310 contain sufficient reagents for 400 reactions of 25µl each. Each system includes:

- 4 × 250µl FFFL, CTTv or GammaSTR® 10X Primer Pair Mix (Fluorescein)
- 4 × 150µl FFFL, CTTv or GammaSTR® Allelic Ladder Mix (Fluorescein)
- 4 × 300µl STR 10X Buffer
- 3µg K562 DNA High Molecular Weight (10ng/µl)
- 2 × 1ml Bromophenol Blue Loading Solution
- 2 × 1ml Blue Dextran Loading Solution
- 250µl Gel Tracking Dye
- 1 × 100µl TH01 Allele 9.3 (Fluorescein), 200 lanes (CTTv system only)
- 1 × 1.2ml Gold ST*R 10X Buffer (FFFL system only)
- 1 Protocol

Storage Conditions: Store all components at –20°C. The fluorescent 10X Primer Pair Mix and fluorescent Allelic Ladder Mix are light-sensitive; therefore, minimize light exposure, and store in the dark. The post-amplification components (allelic ladder, loading solutions and Gel Tracking Dye) are sealed in separate packages to prevent cross-contamination. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc. Store amplified material at –20°C.

2.B. Allelic Ladders and Size Markers

Product	Size	Cat.#
Fluorescent Ladder (CXR), 60–400 Bases	65µl	DG6221

Allelic Ladders

Product	Size	Cat.#
CTTv Allelic Ladder Mix (Fluorescein)[(a)]	150µl	DG2121
FFFL Allelic Ladder Mix (Fluorescein)[(a)]	150µl	DG2131
GammaSTR® Allelic Ladder Mix (Fluorescein)[(a)]	150µl	DG3291

The Fluorescent Ladder (CXR), 60–400 Bases, is a size marker composed of 16 evenly spaced DNA fragments labeled with carboxy-X-rhodamine. When this marker is included in each gel lane, the instruments recommended for fluorescence detection are capable of monitoring and correcting lane-to-lane sample migration differences. The Internal Lane Standard 600 contains the same DNA fragments found in the Fluorescent Ladder (CXR) with additional fragments in the range of 425–600 bases.

2.C. *GenePrint*® Fluorescent Sex Identification Systems

Product	Size	Cat.#
GenePrint® Fluorescent Sex Identification System—Amelogenin (Fluorescein)	100 reactions	DC5171
GenePrint® Fluorescent Sex Identification System—Amelogenin (TMR)	100 reactions	DC6171

Not for Medical Diagnostic Use.

The *GenePrint*® Fluorescent Sex Identification System—Amelogenin (Fluorescein) can be amplified independently or simultaneously with the CTTv Multiplex. X-specific and Y-specific chromosome bands will fall between the TPOX and TH01 loci if amplified along with the CTTv Multiplex.

In the *GenePrint*® Fluorescent Sex Identification System—Amelogenin (TMR), the Amelogenin-specific primer is labeled with tetramethylrhodamine (TMR) and can be co-amplified with the PowerPlex® 1.1 System (compatible with the Hitachi FMBIO® Fluorescence Imaging Systems). The CTTv component of the PowerPlex® System is labeled with TMR, so the TMR-labeled Amelogenin is required.

2.D. *GenePrint*® Fluorescent Monoplex Systems

GenePrint® Fluorescent Monoplex Systems containing the specific primer and allelic ladder plus other components sufficient to perform 100 reactions are available by custom order. Contact your local Promega Branch Office or Distributor for ordering information.

3. Before You Begin

3.A. Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality control measures that are not contained in this manual (14,15).

The quality of the purified DNA sample, as well as small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions, can affect the success of PCR amplification. We suggest strict adherence to recommended procedures for amplification, as well as for denaturing gel electrophoresis and fluorescence detection.

STR analysis is subject to contamination by very small amounts of nontemplate human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, setting up amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (e.g., STR 10X Buffer, K562 control DNA and fluorescein-labeled 10X primer pairs) should be stored separately from those used following amplification (e.g., fluorescein-labeled allelic ladders, loading solutions and Gel Tracking Dye). Always include a negative control reaction (i.e., no template) to ensure reagent purity. We highly recommend the use of gloves and aerosol-resistant pipette tips (e.g., ART® tips, Section 13.G).

Some of the reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Table 1 describes the potential hazards associated with such reagents.

Table 1. Hazardous Reagents.

Reagent	Hazard
acrylamide	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
bisacrylamide	toxic, irritant
formamide (STR 2X Loading Solution)	irritant, teratogen
bind silane (methacryloxypropyltrimethoxysilane)	toxic, moisture sensitive
TEMED	corrosive, flammable
urea	irritant
xylene cyanol FF (STR 2X Loading Solution)	irritant

3.B. Matrix Standardization or Spectral Calibration

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 310 and 3100 Genetic Analyzers. A matrix must be generated for each individual instrument.

The PowerPlex® Matrix Standards, 310 (Cat.# DG4640), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 377 DNA Sequencer for all *GenePrint*® Fluorescent STR Systems, except the *GenePrint*® Fluorescent Sex Identification System—Amelogenin, which requires the PowerPlex® Matrix Standards, 310/377 (Cat.# DG3640). For best results, the PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650), should not be used to generate a matrix on the ABI PRISM® 310 Genetic Analyzer.

The PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650), is required for spectral calibration on the ABI PRISM® 3100 Genetic Analyzer for all *GenePrint*® Fluorescent STR Systems, except the *GenePrint*® Fluorescent Sex Identification System—Amelogenin, which requires the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). The PowerPlex® Matrix Standards, 310 (Cat.# DG4640), or PowerPlex® Matrix Standards, 310/377 (Cat.# DG3640), cannot be used to generate a matrix on this instrument.

For protocols and additional information on matrix standardization, see the *PowerPlex® Matrix Standards, 310, Technical Bulletin* #TBD021, which is supplied with Cat.# DG4640. For protocols and additional information on spectral calibration, see the *PowerPlex® Matrix Standards, 3100/3130, Technical Bulletin* #TBD022, which is supplied with Cat.# DG4650. These manuals are available upon request from Promega or online at: **www.promega.com/tbs/**

4. Amplification

The *GenePrint*® Fluorescent STR Systems have been developed for amplification without artifacts using standard *Taq* DNA polymerase. Special enzymes such as AmpliTaq Gold® DNA polymerase are not required for peak performance. However, if using AmpliTaq Gold® DNA polymerase, we recommend using the Gold ST*R 10X Buffer (Cat.# DM2411), instead of the STR 10X Buffer. Currently, the STR 10X Buffer (pH 9.0) is not compatible with AmpliTaq Gold® DNA polymerase because the optimal pH for the modified *Taq* DNA polymerase is pH 8.3. Also, when using AmpliTaq Gold® DNA polymerase, an additional incubation at 95°C for 11 minutes must be incorporated prior to initiating the thermal cycling program.

The following section gives detailed amplification protocols for the *GenePrint*® Fluorescent STR Systems. Thermal cycling protocols for the PerkinElmer Model 480 and GeneAmp® System 9600 and 9700 thermal cyclers are given for each *GenePrint*® System.

Note: Protocol 12 (Table 6) has been developed for optimal performance of the *GenePrint*® Fluorescent STR System—F13A01, FESFPS, F13B, LPL (Fluorescein) with the GeneAmp® PCR System 9600 thermal cycler (16). This protocol uses Gold ST*R 10X Buffer and AmpliTaq Gold® DNA polymerase.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- thermal cycler, Model 480 or GeneAmp® PCR System 9600 or 9700 (PerkinElmer)
- microcentrifuge
- *Taq* DNA polymerase
- Nuclease-Free Water (Cat.# P1193 or equivalent)
- Mineral Oil (Cat.# DY1151 or equivalent)
- 0.5ml microcentrifuge tubes
- 1.5ml microcentrifuge tubes
- aerosol-resistant pipette tips

4.A. Choice of Thermal Cycling Protocol

The CTTv Multiplex and *GenePrint*® Fluorescent Sex Identification System—Amelogenin (Fluorescein) are optimized for use with PerkinElmer GeneAmp® reaction tubes and PerkinElmer Model 480 thermal cycler. The GammaSTR® Multiplex and *GenePrint*® Fluorescent Sex Identification System—Amelogenin (TMR) are optimized for use with the GeneAmp® PCR System 9600 thermal cycler. The FFFL Multiplex optimized for use with the GeneAmp® PCR System 9700 thermal cycler. However, each system may be used with any of these thermal cyclers.

Please refer to Tables 2, 3 and 4 for recommended and alternative protocols for each system and thermal cycler. Table 5 describes the special template requirement of each multiplex system for use with various detection instruments when using protocol #10. Many customers prefer protocol #10 because it uses the GeneAmp® PCR System 9600 thermal cycler with a heated thermal cycler lid, MicroAmp® reaction tubes and no mineral oil. Specific details for each protocol, including number of cycles, incubation temperatures and times, and ramp times, are provided in Table 6.

When using a thermal cycler for which a system was not optimized, there may be a small loss in product yield or sensitivity, and the balance between loci may change slightly in the multiplex systems. Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 11.

Table 2. Protocol Options for the Model 480 Thermal Cycler.

GenePrint® STR System	Recommended Protocol[1]	Alternative Protocol[2]
CTTv Multiplex	7	1
CTTv Multiplex with Amelogenin[3]	7	1
FFFL Multiplex	7	1
GammaSTR® III Multiplex	7	NA
Amelogenin, CSF1PO, F13A01, TH01 or TPOX	2	1
D16S539, D7S820, D13S317 or D5S818	7	NA
F13B or FESFPS	1	NA
LPL or vWA	7	1

Table 3. Protocol Options for the GeneAmp® PCR System 9600 Thermal Cycler.

GenePrint® STR System	Recommended Protocols[1]	Alternative Protocols[2]
CTTv Multiplex	8,9 or 10[4]	3,4,11
CTTv Multiplex with Amelogenin[3]	8,9 or 10[4]	3,4,11
FFFL Multiplex (using AmpliTaq® DNA polymerase)	8,9 or 10[4]	3,4,11
FFFL Multiplex (using AmpliTaq Gold® DNA polymerase)	12	NA
GammaSTR® III Multiplex	10[4]	9
Amelogenin, CSF1PO, F13A01, F13B, FESFPS, TH01 or TPOX	3,4	NA
D16S539, D7S820, D13S317 or D5S818	9 or 10[4]	NA
LPL or vWA	8,9	3,4

Table 4. Protocol Options for the GeneAmp® PCR System 9700 Thermal Cycler.

GenePrint® STR System	Recommended Protocol
CTTv Multiplex	13
CTTv Multiplex with Amelogenin[3]	13
FFFL Multiplex	13
GammaSTR® III Multiplex	13
Amelogenin, CSF1PO, F13A01, F13B, FESFPS, TH01 or TPOX	13
D16S539, D7S820, D13S317 or D5S818	13
LPL or vWA	13

NA = Not applicable.

[1]Recommended protocols offer similar performance characteristics.

[2]Alternative protocols also work but may trade off performance characteristics, such as greater speed or convenience, for less sensitivity.

[3]The amplification of 25ng or more of K562 DNA using the CTTv system with Amelogenin may result in extra bands at 338, 254 and 161 bases.

[4]Special template requirements for use of protocol #10 with certain multiplex system and detection instrument combinations are described in Table 5.

Table 5. Recommended Amounts of Template For Various Instruments Using Protocol #10.

	Fluorescent STR System		
	CTTv	FFFL	GammaSTR®
Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems	5ng	2–5ng	1ng
ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 and 3100 Genetic Analyzers	1ng	1ng	1ng

Table 6. Amplification Protocols.

Protocol Number	Thermal Cycler[1]	Initial Incubation[2]	Programmed Ramp Times	Cycling for First 10 Cycles	Programmed Ramp Times	Cycling for Last 20 Cycles	Extension Step	Hold Step
1 (Refer to Note 1)	480	96°C for 2 minutes	None	94°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	4°C
2 (Refer to Note 1)	480	96°C for 2 minutes	None	94°C, 1 minute 64°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 64°C, 1 minute 70°C, 1.5 minutes	None	4°C
3 (Refer to Note 2)	9600	96°C for 2 minutes	None	94°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	4°C
4 (Refer to Note 3)	9600	96°C for 2 minutes	50 seconds to 94°C, 1 minute 34 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		45 seconds to 90°C, 1 minute 30 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		None	4°C
5	Protocol 5 is no longer recommended.							
6	Protocol 6 is no longer recommended.							
7 (Refer to Note 1)	9600	96°C for 2 minutes	None	94°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	60°C for 30 minutes	4°C
8 (Refer to Note 2)	9600	96°C for 2 minutes	None	94°C, 1minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	60°C for 30 minutes	4°C
9 (Refer to Note 3)	9600	96°C for 2 minutes	50 seconds to 94°C, 30 seconds 34 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		45 seconds to 90°C, 1 minute 30 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		60°C for 30 minutes	4°C
10 (Refer to Note 4)	9600	96°C for 1 minute	Default ramp to 94°C, 30 seconds 68 seconds to 60°C, 30 seconds 50 seconds to 70°C, 45 seconds		Default ramp to 90°C, 30 seconds 60 seconds to 60°C, 30 seconds 50 seconds to 70°C, 45 seconds		60°C for 30 minutes	4°C
11 (Refer to Note 4)	9600	96°C for 2 minutes	50 seconds to 94°C, 1 minute 34 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		45 seconds to 90°C, 1 minute 30 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		60°C for 30 minutes	4°C
Protocol Number	**Thermal Cycler[1]**	**Initial Incubation[2]**	**Programmed Ramp Times**	**Cycling for First 10 Cycles**	**Programmed Ramp Times**	**Cycling for Last 22 Cycles[3]**	**Extension Step**	**Hold Step**
12 (Refer to Note 4)	9600	96°C for 2 minutes	50 seconds to 94°C, 1 minute 30 seconds to 64°C, 1 minute 15 seconds to 70°C, 1.5 minutes		45 seconds to 90°C, 1 minute 26 seconds to 64°C, 1 minute 15 seconds to 70°C, 1.5 minutes		None	4°C
13 (Refer to Notes 4 and 5)	9700	96°C for 1 minute	ramp 100% to 94°C, 30 seconds ramp 29% to 60°C, 30 seconds ramp 23% to 70°C, 45 seconds		ramp 100% to 90°C, 30 seconds ramp 29% to 60°C, 30 seconds ramp 23% to 70°C, 45 seconds		60°C for 30 minutes	4°C

[1]480 refers to the PerkinElmer model 480 thermal cycler, 9600 refers to the GeneAmp® PCR System 9600 thermal cycler and 9700 refers to the GeneAmp® PCR System 9700 thermal cycler.

[2]Initial incubation performed using AmpliTaq® DNA polymerase. When using AmpliTaq Gold® DNA polymerase, include an additional incubation at 95°C for 11 minutes prior to initiation of the thermal cycling program with any protocol. Also when using AmpliTaq® DNA polymerase, be sure to use the Gold ST*R 10X Buffer.

[3]Please note that Protocols 12 and 13 incorporates two additional cycles in the last portion of the cycling protocol.

Notes for Table 6:

1. Use GeneAmp® reaction tubes, and overlay all reactions with mineral oil.
2. Use GeneAmp® reaction tubes in combination with the GeneAmp® thin-walled tray. This reduces the maximum number of simultaneous reactions to 48 due to the spacing of holes in the tray. Add mineral oil to all reactions.
3. Use MicroAmp® reaction tubes in the MicroAmp® 9600 tray. This allows a maximum of 96 simultaneous reactions. Add mineral oil to all reactions.

   Do not cover the reactions with the system 9600 thermal cycler lid. Cover the reaction tubes loosely with aluminum foil.

   Optional: Add BSA Fraction V (final concentration 60µg/ml) to all reactions. This may result in a slight increase in yield. We recommend Sigma BSA (Cat.# A2153). Performance may vary depending on the source of this component.
4. See Table 5 for recommended amounts of template.

   Use MicroAmp® reaction tubes in the MicroAmp® 9600 tray. This allows a maximum of 96 simultaneous reactions. No mineral oil is needed.

   Cover reactions with the thermal cycler lid.

   Optional: Add BSA Fraction V (final concentration 60µg/ml) to all reactions. This may result in a slight increase in yield. We recommend Sigma BSA (Cat.# A2153). Performance may vary depending on the source of this component.
5. When using the GeneAmp® PCR System 9700 thermal cycler, the ramp rates indicated in the cycling program must be set, and the program must be run in 9600 ramp mode.

   The ramp rates are set in the Ramp Rate Modification screen. While viewing the cycling program, navigate to the Ramp Rate Modification screen by selecting "More", then "Modify". On the Ramp Rate Modification screen the default rates for each step are 100%. The rate under each hold step is the rate at which the temperature will change to that hold temperature. Figure 1 shows the ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

   The ramp mode is set after “start” has been selected for the thermal cycling run. A Select Method Options screen appears. Select 9600 ramp mode, and enter the reaction volume.

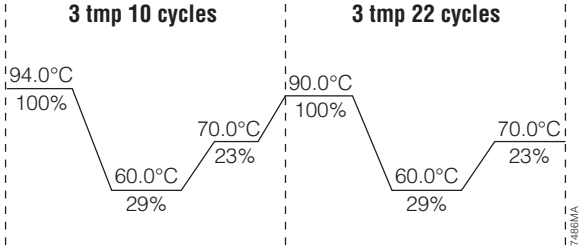


Figure 1. The ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

4.B. Amplification Setup

The use of gloves and aerosol-resistant pipette tips (see Section 13.G) is highly recommended to prevent cross-contamination.

Alternative steps are included in the following procedure for laboratories using the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems and ABI PRISM® 377 DNA Sequencer, and ABI PRISM® 310 and 3100 Genetic Analyzers.

1. Thaw the STR 10X Buffer and 10X Primer Pair(s), and place on ice.

   Note: Mix the STR 10X Buffer and 10X Primer Pair by vortexing each tube for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix, as this may cause the primers to be concentrated at the bottom of the tube.

2. Place one clean, autoclaved 0.5ml reaction tube for each reaction into a rack, and label appropriately.

   Note: If using the GeneAmp® PCR System 9600 thermal cycler, refer to the notes for Table 6 for tube selection.

3. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does waste a small amount of each reagent, it ensures that you will have enough PCR master mix for all samples.

4. Calculate the required amount of each PCR master mix component (Table 7). Multiply the volume (µl) per sample by the total number of reactions (from Step 3) to obtain the final volume (µl).

   Note: The CTTv Multiplex and Amelogenin locus can be amplified simultaneously.

5. In the order listed in Table 7, add the final volume of each reagent to a sterile tube. Mix well, and place on ice.

   Note: If the final volume of *Taq* DNA polymerase added to the master mix is less than 0.5µl, you may wish to dilute the enzyme with STR 1X Buffer first and add a larger volume. The amount of sterile water should be adjusted accordingly so that the final volume per reaction is 25µl. Do not store diluted *Taq* DNA polymerase.

Table 7. PCR Amplification Reaction Setup.

Multiplex Reactions Containing Four Loci

PCR Master Mix Component	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water	17.30				
STR 10X Buffer[1]	2.50				
Multiplex 10X Primer Pair Mix	2.50				
Taq DNA polymerase (at 5u/µl)[1]	0.2 (1.0u)				
total volume	22.50				

Combined CTTv Multiplex and Amelogenin Reactions

PCR Master Mix Component	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water	16.25				
STR 10X Buffer[1]	2.50				
CTTv 10X Primer Pair Mix	2.50				
Amelogenin (Fluorescein) 10X Primer Pair[2]	1.0				
Taq DNA polymerase (at 5u/µl)[1]	0.25 (1.25u)				
total volume	22.50				

Monoplex or Amelogenin-Only Reactions

PCR Master Mix Component	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water	17.45				
STR 10X Buffer[1]	2.50				
locus-specific 10X primer pair	2.50				
Taq DNA polymerase (at 5u/µl)[1]	0.05 (0.25u)				
total volume	22.50				

[1]The volume given assumes a *Taq* DNA polymerase concentration of 5u/µl. For different enzyme concentrations, the volume of enzyme added must be adjusted accordingly. If using AmpliTaq Gold® DNA polymerase, use the Gold ST*R 10X Buffer (instead of the STR 10X Buffer).

[2]Use of more Amelogenin primer has produced extra bands below the expected 212- and 218-base fragments with some samples using protocol #7, especially when 25ng or more of template are used. Amelogenin (TMR) is only for use with the PowerPlex® Systems and should not be used with the CTTv Multiplex System.

4.B. Amplification Setup (continued)

6. Add 22.5µl of PCR master mix to each tube, and place on ice.

Failure to keep the reagents and samples on ice can produce imbalanced amplification of multiplexed loci. If using AmpliTaq Gold® DNA polymerase, it is not necessary to keep the reactions on ice.

7. **Hitachi FMBIO® Users:** Pipet 2.5µl (1–25ng template DNA) of each sample into a tube containing 22.5µl of PCR master mix.

   ABI PRISM® 377 DNA Sequencer, ABI PRISM® 310 Genetic Analyzer, and ABI PRISM® 3100 Genetic Analyzer Users: Use only 1–2ng template DNA.

   Protocol #10 Users: See Table 5 for the amount of template DNA to use with each instrument and *GenePrint*® system.

   Note: If the template DNA is stored in TE buffer (10mM Tris-HCl, 1mM EDTA [pH 7.5]), the volume of the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl) or available magnesium concentration (due to chelation by EDTA). DNA samples stored (or diluted) in sterile, deionized water are not subject to this caution but may contain other PCR inhibitors at low concentrations.

8. **Hitachi FMBIO® Users:** Pipet 2.5µl (25ng) of K562 DNA into a 0.5ml microcentrifuge tube containing 22.5µl of PCR master mix as a positive amplification control.

   ABI PRISM® 377 DNA Sequencer, ABI PRISM® 310 Genetic Analyzer, and ABI PRISM® 3100 Genetic Analyzer Users: Use only 1–2ng of K562 template DNA as a positive amplification control.

9. Pipet 2.5µl of sterile water (instead of template DNA) into a 0.5ml microcentrifuge tube containing 22.5µl of PCR master mix as a negative amplification control.

10. If recommended by the cycling protocol, add 1 drop of mineral oil to each tube. Close the tubes.

    Note: Allow the mineral oil to flow down the side of the tube and form an overlay to limit sample loss or cross-contamination due to splattering.

11. Centrifuge the samples briefly to bring the aqueous contents to the bottom of the tube.

4.C. Amplification Thermal Cycling

1. Place the tubes in a thermal cycler.
2. Select and run a recommended protocol from Table 2, 3 or 4 (Section 4.A).
3. After completion of the thermal cycling protocol, store the samples at –20°C in a light-protected box.

   Note: Storage of amplified samples at 4°C or above may produce degradation products.

5. Polyacrylamide Gel Preparation

Gel preparation depends on the type of instrument used for fluorescent detection. Outlined below are procedures to prepare gels for the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems and ABI PRISM® 377 DNA Sequencer. If you are using a different instrument, please refer to the manufacturer's recommendations.

New glass plates should be soaked in 10% NaOH for 1 hour, then rinsed thoroughly with deionized water before use. New plates should also be etched with a diamond pencil in the corner of one side to distinguish the sides of the plates in contact with the gel.

5.A. Gel Preparation for the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems

There are two size options for gels on the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems, either 32cm × 19cm × 0.4mm (h × w × thickness) or 43cm × 19cm × 0.4mm. The 43cm × 19cm × 0.4mm low-fluorescence glass plates are strongly recommended for better separation. The use of the longer glass plates enables the instrument to distinguish one-base-pair differences and differences in alleles over 300bp more easily. If the Hitachi STaRCall™ Software is used to identify alleles, use square-tooth combs to maximize software performance. Square-tooth combs provide better separation between the lanes. If allelic ladders are used to make allele determinations visually, use either a sharkstooth or a square-tooth comb.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- 40% acrylamide:bis (19:1) and TEMED
- 10X TBE Buffer (Cat.# V4251)
- 10% Ammonium Persulfate (Cat.# V3131)
- Urea (Cat.# V3171)
- bind silane (methacryloxypropyltrimethoxysilane) if square-tooth combs are to be used
- 0.5% acetic acid in 95% ethanol
- Nalgene® tissue culture filter (0.2 micron)
- 32cm × 19cm × 0.4mm (h × w × thickness) low fluorescence glass plates (MiraiBio)
- spacers for SA-32 low fluorescence glass plates
- 43cm × 19cm × 0.4mm (h × w × thickness) low-fluorescence glass plates (Whatman Biometra)
- SA-43 Spacer Set (Whatman Biometra)
- SA-43 Extension (Whatman Biometra) for use with SA-43 glass plates
- power supply
- polyacrylamide gel electrophoresis apparatus for gels ≥30cm
- glass plates and side spacers for polyacrylamide gel ≥30cm
- 14cm vinyl doublefine sharkstooth comb(s), 49 point, 0.4mm thick; or square-tooth comb, 35cm, 60 wells (cut in half for 30 wells/gel), 0.4mm thick (Owl Scientific Cat.# S2S-60A)

5.A. Gel Preparation for the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems (continued)

Materials to Be Supplied by the User (continued)

- Liqui-Nox® detergent (Use of Liqui-Nox® detergent is extremely important, as other kinds of detergent can build up on the glass plates.)
- clamps (e.g., large office binder clips)
- diamond pencil for marking glass plates

Unpolymerized acrylamide is a neurotoxin and suspected carcinogen; avoid inhalation and contact with skin. Read the warning label, and take the necessary precautions when handling this substance. Always wear gloves and safety glasses when working with acrylamide powder or solutions.

Hitachi FMBIO® Fluorescence Imaging Systems

1. Thoroughly clean the shorter and longer glass plates twice with 95% ethanol and Kimwipes® tissues.

   Note: The plates require bind silane treatment if using a square-tooth comb (see below). The plates do not require a special bind silane treatment when using a sharkstooth comb.

   Bind Silane Treatment of Glass Plate

   Prepare fresh binding solution in a chemical fume hood. Add 1.5µl of bind silane to a 1.5ml microcentrifuge tube containing 0.5ml of 0.5% acetic acid in 95% ethanol. Wipe the etched side of the shorter glass plate in the comb region using a Kimwipes® tissue saturated with the freshly prepared binding solution. Wait 5 minutes for the binding solution to dry. Wipe the shorter glass plate 3–4 times with 95% ethanol and Kimwipes® tissues in the comb area to remove the excess binding solution.

2. Assemble the glass plates by placing 0.4mm side spacers between the plates and using clamps to hold them in place (3–4 clamps on each side). A bottom spacer is neither required nor recommended. Place the assembly horizontally on a test tube rack or similar support.

3. Prepare a 4% or 6% acrylamide solution (total of 30ml for a 32cm plate or 45ml for a 43cm plate) by combining the ingredients listed in Table 8.

Table 8. Preparation of 4% and 6% Polyacrylamide Gels.

Component	4% Gel (32cm)	4% Gel (43cm)	6% Gel (32cm)	6% Gel (43cm)	Final Concentration
urea	12.6g	18.9g	12.6g	18.9g	7M
deionized water	16.0ml	24.0ml	14.5ml	21.75ml	–
10X TBE buffer	1.5ml	2.25ml	1.5ml	2.25ml	0.5X
40% acrylamide:bis (19:1)	3.0ml	4.5ml	4.5ml	6.75ml	4% or 6%
total volume	30.0ml	45.0ml	30.0ml	45.0ml	

Note: If preparing multiple gels on a daily basis, a larger 4% or 6% stock solution may be prepared, filtered as in Step 4 below and stored at 4°C in the dark for up to one month. To prepare a single gel, remove either 30ml or 45ml of the stock solution, and continue with Step 6.

4. Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene®

Component	32cm gel (30ml)	43cm gel (45ml)
TEMED	20µl	30µl
10% ammonium persulfate	200µl	300µl

tissue culture filter).

5. Slowly pour the filtered acrylamide solution into a squeeze bottle.
6. Add the following amounts of TEMED and 10% ammonium persulfate, and mix gently.
7. Pour the gel by starting at the well end of the plates. Carefully pour the acrylamide between the horizontal glass plates. Allow the solution to fill the top width of the plates. Slightly tilt the plates to assist the movement of the solution to the bottom of the plates while maintaining a constant flow of the solution. When the solution begins to flow out from the bottom, position the plates horizontally.
8. Insert the straight side of a 14cm doublefine (49 point) sharkstooth comb (6mm of the comb should be between the two glass plates). If using a square-tooth comb, insert the comb between the glass plates until the teeth are almost completely inserted into the gel.
9. Secure the comb with three evenly spaced clamps.
10. Pour the remaining acrylamide solution into a disposable conical tube as a polymerization control. Rinse the squeeze bottle, including the spout, with water.
11. Allow polymerization to proceed for at least 1 hour. Check the polymerization control (Step 10) to be sure that polymerization has occurred.

    Note: The gel may be stored overnight if a paper towel saturated with deionized water and plastic wrap are placed around the top and bottom to prevent the gel from drying out (crystallization of the urea will destroy the gel).

5.B. Gel Preparation for the ABI PRISM® 377 DNA Sequencer

When working with the glass plates for the ABI PRISM® 377 DNA Sequencer, it is extremely important to avoid contact between the gel side of the plates and paper towels. Rinse the plates extremely well with deionized water, and allow to air-dry in a dust-free environment before use.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- Long Ranger® gel solution (Cambrex Cat.# 50611)
- 10X TBE Buffer (Cat.# V4251)
- 10% Ammonium Persulfate (Cat.# V3131)
- TEMED
- Urea (Cat.# V3171)
- Nalgene® tissue culture filter (0.2 micron)
- 36cm front and rear glass plates (refer to the instrument manual for recommendations)
- 36cm gel spacers (0.2mm thick)
- 36-well sharkstooth comb or 34-well square-tooth comb (0.2mm thick)
- clamps
- Liqui-Nox® detergent (Use of Liqui-Nox® detergent is extremely important, as other kinds of detergent can build up on the glass plates.)

The following protocol is for the preparation of a 36cm denaturing polyacrylamide gel for use with the ABI PRISM® 377 DNA Sequencer. Low-fluorescence glass plates are recommended and may be obtained from the instrument manufacturer.

1. Thoroughly clean the glass plates with hot water and a 1% Liqui-Nox® solution. Rinse extremely well using deionized water. Allow the glass plates to air-dry.

2. Assemble the glass plates by placing 0.2mm side gel spacers between the front and rear glass plates. Hold the plates together using binder clamps (4 clamps on each side). Place the assembly horizontally on a test tube rack or similar support.

3. Prepare a 5% Long Ranger® acrylamide gel (total of 50ml) by combining the ingredients listed in Table 9. Stir the solution until the urea has dissolved.

Table 9. Preparation of a 5% Long Ranger® Polyacrylamide Gel.

Component	5% Gel	Final Concentration
urea	18g	6M
deionized water	26ml	–
10X TBE buffer	5.0ml	0.5X
50% Long Ranger® gel solution	5.0ml	5%
total volume	**50.0ml**	

4. Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene® tissue culture filter), and de-gas for an additional 5 minutes.

5. Add 35µl of TEMED and 250µl of 10% ammonium persulfate to 50ml of acrylamide solution, and mix gently.

6. Using a disposable 30cc syringe, pour the gel by starting at the well end of the plates and carefully injecting the acrylamide solution between the horizontal glass plates. Allow the solution to fill the top width of the plates. While maintaining a constant flow of solution, gently tap the glass plates to assist the movement of solution to the bottom of the plates.

7. Insert the straight edge of one 36-well sharkstooth comb, or insert a 34-well square-tooth comb between the glass plates.

8. Secure the comb with three evenly spaced clamps.

9. Pour the remaining acrylamide solution into a disposable conical tube as a polymerization control.

10. Allow polymerization to proceed for at least 2 hours. Check the polymerization control (Step 9) to ensure that polymerization has occurred.

    Note: The gel may be stored overnight if a paper towel saturated with 1X TBE and plastic wrap are placed around the top and bottom of the gel to prevent the gel from drying out (crystallization of the urea will destroy the gel).

6. Polyacrylamide Gel Electrophoresis and Detection

Electrophoresis protocols depend on the type of instrument used for fluorescence detection. The following sections provide procedures for loading and running gels on the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems (Section 7), ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 Genetic Analyzer, (Section 8) and ABI PRISM® 3100 Genetic Analyzer (Section 9). If a different instrument is used for detection, please refer to the manufacturer's recommendations for that particular instrument.

The Fluorescent Ladder

The Fluorescent Ladder (CXR), 60–400 Bases, contains 16 evenly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375 and 400 bases in length. This ladder may be used as an internal size standard in each lane to increase precision in analyses. Inclusion of the Fluorescent Ladder (CXR) in each lane reduces the number of allelic ladder lanes needed per gel and, therefore, increases the number of lanes available for samples. Allelic ladders still need to be run on one or two lanes of every gel as a control to verify that the gel ran correctly and that the allele sizes are correct. Be aware that the sizes determined by the software may not exactly correspond to the sequenced sizes given in Table 13 because samples migrate on a gel according to length, sequence and dye label.

6. Polyacrylamide Gel Electrophoresis and Detection (continued)

The Fluorescent Ladder (CXR), 60–400 Bases, is required when using the ABI PRISM® 377 DNA Sequencer, ABI PRISM® 310 Genetic Analyzer or ABI PRISM® 3100 Genetic Analyzer. The Genescan® software provided with these instruments requires use of a sizing ladder. The Fluorescent Ladder (CXR) must be included in all lanes to account for lane-to-lane or run-to-run variability. Adjustments in the allele sizes will be made from lane to lane when the allele size is compared to the Fluorescent Ladder (CXR) contained within each lane.

When using the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems, inclusion of the Fluorescent Ladder (CXR) is optional. If alleles are called visually by comparing the sample alleles directly against the allelic ladders, the Fluorescent Ladder (CXR) is not needed. However, if the Hitachi STaRCall™ Software is used for identifying alleles, we recommend including the Fluorescent Ladder (CXR).

Note: The Internal Lane Standard 600 (Cat.# DG1071) contains the same DNA fragments as the Fluorescent Ladder (CXR), 60–400 Bases, with additional DNA fragments of 425, 450, 475, 500, 550 and 600 bases.

7. Sample Preparation, Gel Electrophoresis and Detection on the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems

7.A. Gel Pre-Run

1. Remove the clamps from the polymerized acrylamide gel, and clean the glass plates with paper towels saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb, and remove the comb.
3. Add 0.5X TBE Buffer to the bottom chamber of the electrophoresis apparatus.
4. Gently lower the gel (glass plates) into the bottom chamber with the longer plate facing out and the well-side on top.
5. Secure the glass plates to the sequencing gel apparatus.
6. Add 0.5X TBE Buffer to the top chamber of the electrophoresis apparatus.
7. Use a 50–100cc syringe filled with buffer to remove any air bubbles on top of the gel. Be certain the well area is devoid of air bubbles and small pieces of polyacrylamide. Use a syringe with a bent 19-gauge needle to remove air bubbles from the bottom of the gel.
8. Pre-run the gel to achieve a surface temperature of approximately 50°C. Consult the manufacturer's instruction manual for recommended electrophoresis conditions.

   Note: As a reference, we generally use 40–45 watts for 30 minutes for a 32cm gel or 60–65 watts for 30 minutes for a 43cm gel. The gel running conditions may have to be adjusted to reach a temperature of 50°C.

7.B. Sample Preparation, Loading and Electrophoresis

Use of the Fluorescent Ladder (CXR) is optional with the Hitachi instruments. If alleles are called visually, we recommend running an allelic ladder in every third lane so that each sample is next to a ladder.

1. If the Fluorescent Ladder (CXR) is not used, prepare the PCR samples or allelic ladders by combining 2.5µl of Bromophenol Blue Loading Solution with 2.5µl of PCR sample or allelic ladder.

   If the Fluorescent Ladder (CXR) is used, prepare PCR samples and allelic ladders by combining 1µl of Fluorescent Ladder (CXR), 3µl of Bromophenol Blue Loading Solution and 2µl of sample or allelic ladder [we recommend running two lanes of allelic ladder per gel when using the Fluorescent Ladder (CXR)].

   Note: The Bromophenol Blue Loading Solution does not contain xylene cyanol because it fluoresces and is detected by the FMBIO® instruments.

2. After samples are prepared, centrifuge the tubes briefly to bring the contents to the bottom of the tube.

3. **Optional:** Place 6µl of Gel Tracking Dye in one tube. The Gel Tracking Dye contains both bromophenol blue and xylene cyanol. This dye may be loaded in the outermost lane of the gel and used as a visual indicator of migration. We recommend leaving two empty lanes between the gel tracking dye and sample lanes so that the xylene cyanol fluorescence does not interfere with sample interpretation.

   Notes:

   1. To analyze the CTTv Multiplex with Amelogenin reactions, mix the corresponding ladders 1:1 before mixing with loading solution. The number of ladder lanes depends on personal preference and the number of samples analyzed.
   2. To use the TH01 allele 9.3 alone, mix 0.5µl of allele 9.3 with 1.5µl of 1X STR Buffer before mixing with the loading solution. To use in combination with the TH01 monoplex or CTTv Allelic Ladder, mix 0.5µl of allele 9.3 with 2.0µl of allelic ladder before mixing with the appropriate loading dye.

4. Denature samples by heating at 95°C for 2 minutes, and immediately chill on crushed ice or in an ice-water bath.

   Denature samples just prior to loading the gel. Sample DNA will re-anneal if denatured hours before loading. This may produce fragments of indeterminate migration.

5. If using a sharkstooth comb, flush the urea from the well area with a 50–100cc syringe filled with buffer. Carefully insert the comb teeth into the gel approximately 1–2mm. Leave the comb inserted in the gel during gel loading and electrophoresis. If a square-tooth comb is used, clean the individual wells with buffer using a 50–100cc syringe, and do not reinsert the comb. The samples will be loaded directly into the wells.

7.B. Sample Preparation, Loading and Electrophoresis (continued)

6. Load 3µl of each sample into the respective wells. We recommend using gel loading tips to load the wells formed by the square-tooth combs. The loading process should take no longer than 20 minutes to prevent the gel from cooling.

7. When loading is complete, run the gel using the same conditions as in Section 7.A (gel pre-run).

   Note: In 6% gels, bromophenol blue migrates at approximately 25 bases, and xylene cyanol migrates at approximately 105 bases. In 4% gels, bromophenol blue migrates at approximately 40 bases, and xylene cyanol migrates at approximately 170 bases.

8. Use the size ranges for each locus (see Table 13, Section 13.B) and the migration characteristics of the dyes (see Step 7) to stop electrophoresis any time after the locus of interest has passed the midpoint of the gel. If running more than one locus or a multiplex, be careful not to run the smallest locus off the bottom of the gel.

7.C. Detection

1. After electrophoresis, remove the gel/glass plate unit from the apparatus. Remove the comb and side spacers, but do not separate the glass plates.

2. The plates must be very clean for scanning. Clean both sides of the gel/glass plate unit with deionized water and paper towels. Do not use ethanol to clean the plates. The ethanol fluoresces and is detected by the FMBIO® instruments.

3. Scan the gel according to the parameters listed in Table 10. Use the 505nm filter to detect fluorescein-labeled fragments and the 650nm filter to detect the Fluorescent Ladder (CXR), 60–400 Bases. Different laboratories may wish to modify these parameters according to their specific preferences.

   Note: If the signal is too intense, dilute samples in 1X STR Buffer before mixing with loading solution or use less DNA template in the amplification reactions.

7.D. Data Analysis

Controls

Observe the lanes containing negative controls. They should be devoid of amplification products.

Observe the lanes containing K562 DNA positive controls. Compare the K562 alleles with the locus-specific ladder. The expected K562 alleles for each locus are listed in Table 13. Figure 2 (Section 10) shows an example of results obtained after amplification of the positive control K562 DNA using the *GenePrint*® Multiplex CTTv, FFFL and GammaSTR® Systems. The K562 DNA contains imbalanced alleles at several loci. This result is due to the unusual chromosome content of this cell line and is not a function of the *GenePrint*® Fluorescent STR System performance.

Table 10. Instrument Parameters for the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems.

Parameter	FMBIO® Fluorescence Imaging System	FMBIO® II Fluorescence Imaging System
Material Type	Acrylamide gel	Acrylamide gel
Resolution:		
Horizontal	150dpi	150dpi
Vertical	150dpi	150dpi
Rate	0.1024s/line	NA
Repeat	1 time	256 times
Gray Level Correction Type	Range	Range
Cutoff Threshold:		
Low (Background)	50%	50%
High (signal)	1%	1%
Reading Sensitivity	80%	100% (505nm channel) 100% (650nm channel)
Focusing Point	NA	0mm

NA = Not applicable.

Representative STR Data

Representative STR data obtained using the Hitachi FMBIO® II Fluorescent Scanner and *GenePrint*® Fluorescent STR Multiplex—GammaSTR® are shown in Figure 3 (Section 10).

Allelic and Fluorescent Ladders

In general, allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. The allelic ladder sizes and repeat units are listed in Table 13. Visual comparison between the allelic ladder and amplified samples of the same locus allows precise assignment of alleles. Analysis using specific instrumentation also allows allele determination by comparing amplified sample fragments with either allelic ladders, internal size standards or both (see software documentation from instrument manufacturer). When using an internal size standard, the calculated lengths of allelic ladder components will differ from those listed in Table 13. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and those of the internal size standard.

7.E. Reuse of Glass Plates

Separate the glass plates, and discard the gel. Clean the glass plates with deionized water and a 1% solution of Liqui-Nox® detergent. The use of Liqui-Nox® detergent is extremely important, as other kinds of soap can build up on the glass plates. Buildup will result in low signal and high background on the gels. If the glass plates have a soap residue buildup on them, we recommend soaking in 10% sodium hydroxide for 1 hour and rinsing well in deionized water.

If bind silane is used to fix the gel to the smaller glass plate, soak the plate in 10% sodium hydroxide for 1 hour (or until the gel comes off the plate) before cleaning the glass plates.

8. Sample Preparation, Gel Electrophoresis and Detection on the ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 Genetic Analyzer

Instructions for use with the ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 Genetic Analyzer are given below. Please refer to the user's manuals provided with these instruments for specific operating instructions.

8.A. Instrument Preparation

ABI PRISM® 377 DNA Sequencer

1. Open the GeneScan® data collection software.
2. Prepare a GeneScan® sample sheet as described in the GeneScan® analysis software user's manual.
3. Create a new GeneScan® run using the following settings:

Plate Check Module:	Plate Check A
PreRun Module:	PR GS 36A-2400
Run Module:	GS 36A-2400
Collect Time:	2.25 hours
Well-to-Read Distance:	36cm

4. Select the appropriate sample sheet and comb selection by using the pull-down menus.
5. Select the appropriate gel matrix file created in Section 3.B.

ABI PRISM® 310 Genetic Analyzer

Refer to the ABI PRISM® 310 Genetic Analyzer user's manual for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.

1. Open the ABI PRISM® 310 data collection software.
2. Prepare a GeneScan® sample sheet as described in the ABI PRISM® 310 Genetic Analyzer user's manual. Enter the appropriate sample information in the "sample info" column.
3. Create a new GeneScan® injection list. Select the appropriate sample sheet by using the pull-down menu.
4. Select the "GS STR POP4 (1ml) F" Module using the pull-down menu. Use the settings shown below.

Inj. Secs:	5
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time (min):	24

(!) You may need to optimize the injection time for individual instruments.

5. Select the appropriate gel matrix file created in Section 3.B.
6. To automatically analyze data, select the Auto Analyze checkbox and the appropriate analysis parameters and size standard. Refer to the ABI PRISM® 310 Genetic Analyzer user's manual for specific information on these options.

8.B. Sample Preparation and Loading

The Fluorescent Ladder (CXR), 60–400 Bases, is available as the internal size marker for two-color detection and analysis of amplified samples. With this approach, only 2–3 lanes of allelic ladder are required per gel.

1. Prepare samples according to the instructions given in Table 11.
2. Briefly centrifuge samples to bring the contents to the bottom of the tubes.
3. Denature samples by heating at 95°C for 2 minutes, and immediately chill on crushed ice or in an ice-water bath.

Denature samples just prior to loading the gel. Sample DNA will partially reanneal if denatured hours before loading.

Table 11. Instrument-Specific Instructions for Sample Preparation Using the ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310 Genetic Analyzer.

ABI PRISM® 377 DNA Sequencer
For PCR-amplified samples, combine 1µl of sample with 1.5µl of Blue Dextran Loading Solution and 0.5µl of Fluorescent Ladder (CXR).
For allelic ladders, dilute the ladder 1:10 in 1X STR Buffer, then combine 1µl of diluted ladder with 1.5µl of Blue Dextran Loading Solution and 0.5µl of Fluorescent Ladder (CXR).
ABI PRISM® 310 Genetic Analyzer
For PCR-amplified samples, combine 1µl of sample with 24.5µl of formamide (deionized) and 0.5µl of Fluorescent Ladder (CXR).
For allelic ladders, dilute the ladder 1:10 in 1X STR Buffer, then combine 1µl of diluted ladder with 24.5µl of formamide (deionized) and 0.5µl of Fluorescent Ladder (CXR).

Notes:

1. Samples amplified with the GammaSTR® Multiplex (1–2ng of template) will need to be diluted 1:5 (i.e., 1 part sample:4 parts 1X STR Buffer) before mixing with the loading solution.
2. To analyze the CTTv Multiplex with Amelogenin reactions, mix the corresponding ladders 1:1 before mixing with loading solution. The number of ladder lanes depends on personal preference and the number of samples analyzed.
3. To use the TH01 allele 9.3 alone, mix 0.5µl of allele 9.3 with 1.5µl of 1X STR Buffer before mixing with the loading solution. To use in combination with the TH01 monoplex or CTTv Allelic Ladder, mix 0.5µl of allele 9.3 with 2.0µl of allelic ladder before mixing with the appropriate loading dye.

8.B. Sample Preparation and Loading (continued)

ABI PRISM® 377 DNA Sequencer

1. Pre-run the gel to achieve a surface temperature of approximately 50°C. After the 15–20 minute pre-run, pause the instrument by clicking on Pause.
2. Use a 30cc syringe filled with buffer to flush the urea from the well area.
3. Load 1.5µl of each denatured sample into the respective wells.
4. Place the lid on the upper buffer chamber, and close the instrument door.

ABI PRISM® 310 Genetic Analyzer

1. Assemble the tubes in the appropriate autosampler tray (48-tube or 96-tube).
2. Place the autosampler tray in the instrument, and close the instrument doors.

8.C. Electrophoresis and Detection

ABI PRISM® 377 DNA Sequencer

1. After loading, select Cancel to stop the pre-run. Select Run to begin electrophoresis.
2. Monitor electrophoresis by observing the gel image and status windows.
3. Allow electrophoresis to proceed for 2.25 hours. At this point, the 400-base fragment will have migrated past the laser.
4. Analyze the gel according to the GeneScan® analysis software user's manual.

   Note: If the signal is too intense (a peak height greater than 3,000RFU), dilute the sample in 1X STR Buffer before mixing with loading solution or use less DNA template in the amplification reaction.

ABI PRISM® 310 Genetic Analyzer

1. After loading the sample tray and closing the doors, select Run to start the capillary electrophoresis system.
2. Monitor electrophoresis by observing the raw data and status windows.
3. Each sample will take approximately 30 minutes for syringe pumping, sample injection and sample electrophoresis.
4. Analyze data according to the GeneScan® analysis software user's manual.

   Note: Peak heights outside the linear range of the instrument may generate artifact peaks due to instrument saturation (i.e., overloading the sample). Bleedthrough (pull-ups) from one color to another may be observed. Saturated signal may also appear as two peaks (split peak).

8.D. Data Analysis

Representative STR data obtained using the ABI PRISM® 377 DNA Sequencer, *GenePrint*® Fluorescent STR Multiplex—GammaSTR® and Fluorescent Ladder (CXR), 60–400 Bases, are shown in Figure 4 (Section 10).

Controls

Observe the lanes containing negative controls. They should be devoid of amplification products.

Observe the lanes containing K562 DNA positive control reactions. Compare the K562 alleles with the locus-specific allelic ladder. The expected K562 alleles for each locus are listed in Table 13, Section 13.B.

The K562 DNA contains imbalanced alleles at several loci. This result is due to the unusual chromosome content of the K562 cell line and is not a function of the *GenePrint*® Fluorescent STR Systems.

Allelic Ladders

In general, allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. The allelic ladder sizes and repeat units are listed in Table 13, Section 13.B. Analysis using GeneScan® analysis software allows allele determination by comparing amplified sample fragments with either allelic ladders, internal size standards or both. When using an internal size standard, the calculated lengths of allelic ladder components will differ from those listed in Table 13. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and those of the internal size standard.

8.E. Reuse of Glass Plates

For the ABI PRISM® 377 DNA Sequencer, separate the glass plates, and discard the gel. Clean the plates in the following manner: rinse with hot water, wash with 1% Liqui-Nox® solution, rinse well with hot water, wash with 1N NaOH, rinse extremely well with deionized water and allow the plates to air-dry.

9. Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer and Data Collection Software, Version 1.1

Materials to Be Supplied by the User

- dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- aerosol-resistant pipette tips
- 3100 capillary array, 36cm
- performance optimized polymer 4 (POP-4™) for the 3100
- 10X genetic analyzer buffer with EDTA
- sample tubes and septa for the 3100
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650)

Formamide quality is critical. Use deionized formamide with a conductivity <100µS/cm. Formamide can be dispensed into aliquots and frozen at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Formamide with a conductivity greater than 100µS/cm may contain ions that compete with DNA during injection, resulting in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

9.A. Sample Preparation

The Fluorescent Ladder (CXR), 60–400 Bases (Cat.# DG6221), is available as the internal lane standard for 4-color detection and analysis of amplified sample.

1. Prepare a loading cocktail by combining and mixing the Fluorescent Ladder (CXR), 60–400 Bases, and deionized formamide as follows:

   [(1µl Fluorescent Ladder × (# injections)] + [(9µl deionized formamide) × (# injections)]

2. Pipet 10µl of formamide/fluorescent ladder loading cocktail into each well.
3. Add 1µl of amplified sample.

   Notes:

   1. Samples (1–2ng of template) amplified with the FFFL, CTTv and GammaSTR® Systems will need to be diluted 1:5 (i.e., 1 part sample: 4 parts 1X STR Buffer) before mixing with the loading solution.
   2. To analyze the CTTv Multiplex with Amelogenin reactions, mix the corresponding ladders 1:1 before mixing with loading solution. The number of ladder lanes depends on personal preference and the number of samples analyzed.
   3. To use the TH01 allele 9.3 alone, mix 0.5µl of allele 9.3 with 1.5µl of 1X STR Buffer before mixing with the loading solution. To use in combination with the TH01 monoplex or CTTv Allelic Ladder, mix 0.5µl of allele 9.3 with 2.0µl of allelic ladder before mixing with the appropriate loading dye.
   4. Instrument detection limits vary; therefore, injection time or the amount of product mixed with loading cocktail may need to be increased or decreased. If peak heights are too high (>4,000RFU), the samples can be diluted in the reaction buffer used for amplification

(Gold ST*R 1X Buffer or 1X STR Buffer) before mixing with loading cocktail. This may result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles.

4. For allelic ladders, dilute the ladder 1:5 (i.e., 1 part sample:4 parts 1X STR Buffer), then combine 1µl of diluted ladder with 9.0µl of deionized formamide and 1.0µl of Fluorescent Ladder (CXR). Cover wells with appropriate septa.

5. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature the samples just prior to loading into the ABI PRISM® 3100 Genetic Analyzer.

   Note: Brief centrifugation of prepared samples will remove bubbles that may affect analysis.

9.B. Instrument Preparation

Refer to the ABI PRISM® 3100 Genetic Analyzer user's manual for instructions on cleaning the pump blocks, installing the capillary array, performing a spatial calibration, and adding polymer to the reserve syringe.

1. Open the ABI PRISM® 3100 data collection software.
2. Open a new plate record. Name the plate, and select "GeneScan". Select the plate size (96-well or 384-well). Select "Finish".
3. Complete the plate record spreadsheet for the wells you have loaded.
4. In the "BioLIMS Project" column, select "3100_Project1" from the pull-down menu.
5. In the "Dye Set" column, select "Z" from the pull-down menu.
6. In the "Run Module 1" column, select "GeneScan36_POP4DefaultModule" from the pull-down menu.
7. To collect data without autoanalyzing, select "No Selection" in the "Analysis Module 1" column. Analysis parameters can be applied after data collection and during data analysis using the GeneScan® analysis software.

   To analyze data during data collection, an appropriate analysis module must be selected in the "Analysis Module 1" column. Refer to the ABI PRISM® 3100 Genetic Analyzer user's manual for specific instructions on creating analysis modules.
8. Select "OK". This new plate record will appear in the pending plate records table on the plate setup page of the data collection software.
9. Place samples in instrument, and close the instrument doors.
10. In the pending plate records table, click once on the name of the plate record you just created.

9.B. Instrument Preparation (continued)

11. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples to link the plate to the plate record.
12. When the plate record is linked to the plate, the plate graphic will change from yellow to green, the plate record moves from the pending plate records table to the linked plate records table, and the "Run Instrument" button becomes enabled.
13. Select "Run Instrument" on the toolbar to start the sample run.
14. Monitor electrophoresis by observing the run, status, array and capillary views windows in the collection software. Each run (16 samples/ capillaries) will take approximately 45 minutes.

9.C. Sample Detection

1. Analyze data using the GeneScan® analysis software.
2. Review the raw data for one or more sample runs. Highlight the sample file name, then under the sample menu, select "raw data". Move the cursor so the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [red]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.

   The recommended analysis parameters are:

Analysis Range	Start: Defined in Step 2 Stop: 10,000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light[1]
Peak Detection	Peak Amplitude Thresholds[2]: B: Y: G: R: Min. Peak Half Width: 2pts
Size Call Range	Min: 60 Max: 600
Size Calling Method	Local Southern Method
Split Peak Correction	None

[1]Smoothing options should be determined by individual laboratories. Occasionally the separation control alleles and the TH01 alleles 9.3 and 10 will not be distinguished using heavy smoothing.

[2]The peak amplitude thresholds are the minimum peak height that the software will call as a peak. Values for the peak amplitude thresholds are usually 50–200RFU and should be determined by individual laboratories.

3. The analysis parameters can be saved in the "Params" folder.
4. Apply the stored analysis parameters file to the samples.
5. Assign a new size standard. Select a sample file, and highlight the arrow next to size standard, then select "define new." Assign size standard peaks as shown in Figure 2. Store the size standard in the "SizeStandards" folder.
6. Apply the size standard file to the samples, then analyze the sample files.
7. See Section 9.D for further data analysis.

For additional information regarding the GeneScan® analysis software, refer to the GeneScan® analysis software user's manual.

Notes:

1. Peak heights outside the linear range of the instrument may generate artifact peaks due to instrument saturation (i.e., overloading the sample).
2. If the sample peak heights are not within the linear detection of the instrument, the ratio of stutter peaks to real allele peaks increases and allele designations become difficult to interpret. The balance of the peak heights may also appear less uniform.
3. There may be variation between instruments regarding their relative fluorescent units detected using the same sample.

9.D. Data Analysis

Controls

Observe the lanes containing negative controls. They should be devoid of amplification products.

Observe the lanes containing K562 DNA positive control reactions. Compare the K562 alleles with the locus-specific allelic ladder. The expected K562 alleles for each locus are listed in Table 13, Section 13.B.

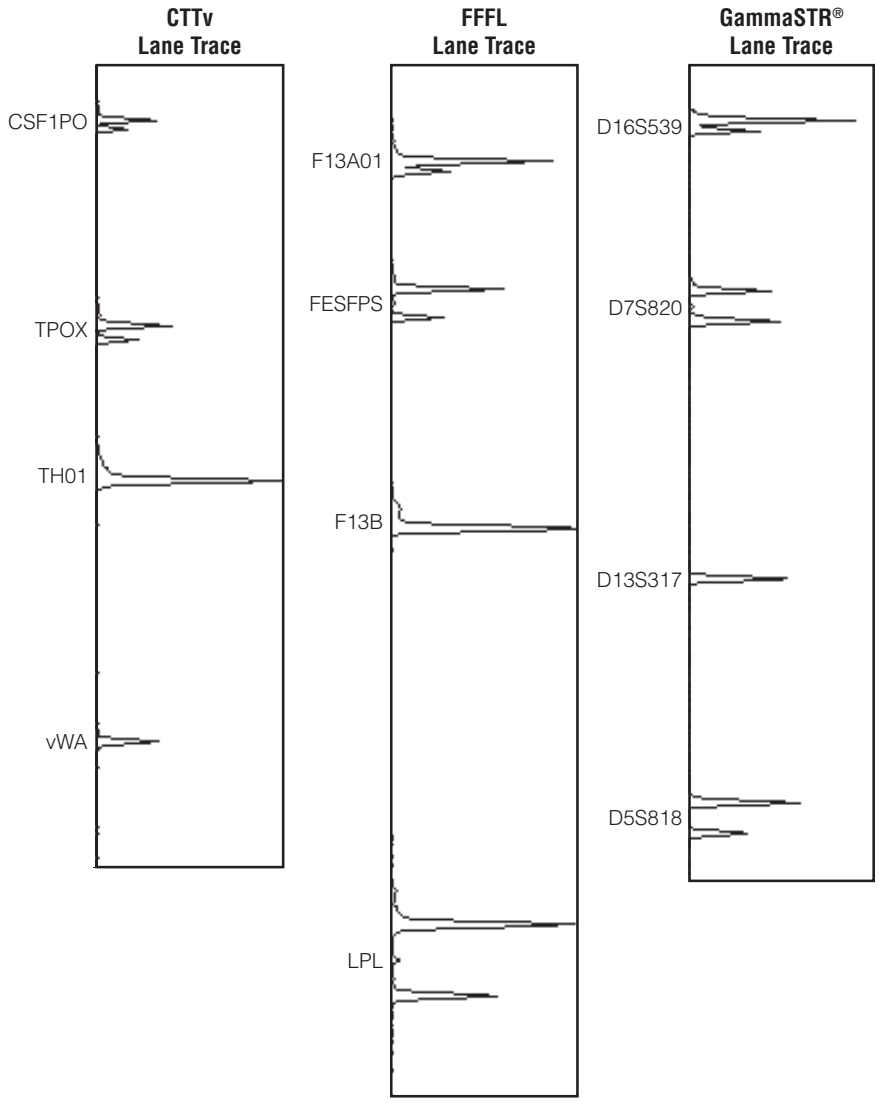
The K562 DNA contains imbalanced alleles at several loci. This result is due to the unusual chromosome content of the K562 cell line and is not a function of the *GenePrint*® Fluorescent STR Systems performance.

Allelic Ladders

In general, allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. The allelic ladder sizes and repeat units are listed in Table 13, Section 13.B. Analysis using GeneScan® analysis software allows allele determination by comparing amplified sample fragments with either allelic ladders, internal size standards or both. When using an internal size standard, the calculated lengths of allelic ladder components will differ from those listed in Table 13. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and those of the internal size standard.

10. Representative STR Data

Representative data are shown in Figures 2, 3 and 4.



Locus	Allele Height (Shorter Peak/ Higher Peak)
CSF1PO	51%
TPOX	53%
TH01	NA
vWA	NA

Locus	Allele Height (Shorter Peak/ Higher Peak)
F13A01	38%
FESFPS	45%
F13B	NA
LPL	50%

Locus	Allele Height (Shorter Peak/ Higher Peak)
D16S539	42%
D7S820	83%
D13S317	NA
D5S818	59%

5857TA

Figure 2. K562 DNA amplified using the *GenePrint*® Fluorescent STR Systems. Five nanograms of K562 DNA were amplified using the *GenePrint*® CTTv and FFFL Multiplex Systems, and one nanogram of K562 DNA was amplified using the GammaSTR® Multiplex System. The amplified DNA was separated on a 43cm, 4% denaturing polyacrylamide gel for 1 hour at 65 watts using the Hitachi FMBIO® Fluorescence Imaging System. Lane traces for K562 DNA amplified using each of the three *GenePrint*® Systems are shown. The table below each lane trace indicates the percent differences in allele height (i.e., peak heights minus typical interlocus background value) occurring as a result of the unusual chromosome content of the K562 cell line. These variations are not a consequence of primer imbalance in amplification.

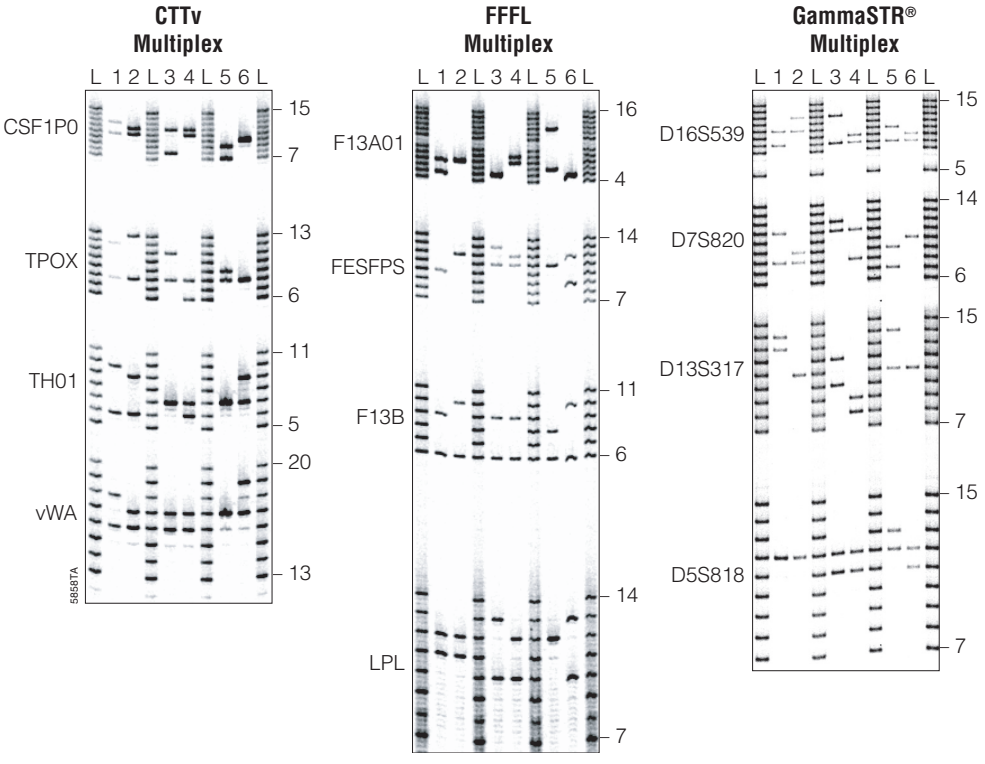


Figure 3. STR analyses performed using the fluorescein-labeled *GenePrint*® STR Multiplex Systems and Hitachi FMBIO® Fluorescence Imaging System. DNA samples amplified using the CTTv, FFFL and GammaSTR® Multiplex Systems are shown. For each system, six DNA samples were amplified (lanes 1–6) and are shown with allelic ladders for the corresponding loci (lanes L). Each allelic ladder is labeled to its right with the number of copies of the repeat sequence contained within the corresponding largest and smallest alleles of each locus. All materials were separated using 4% denaturing polyacrylamide gels. The CTTv, FFFL and GammaSTR® Multiplex Systems were detected using the Hitachi FMBIO® II Fluorescence Imaging System.

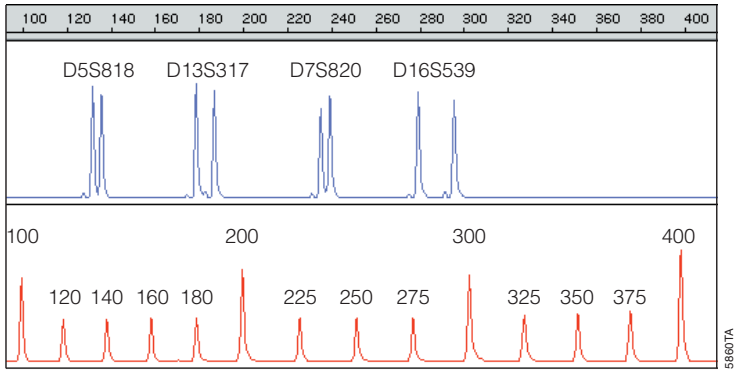


Figure 4. STR analyses performed using the GammaSTR® Multiplex System, Fluorescent Ladder (CXR), 60–400 Bases, and ABI PRISM® 310 Genetic Analyzer. The upper panel shows the alleles of loci D5S818, D13S317, D7S820 and D16S539 amplified using the GammaSTR® System and 1ng DNA template. The sample was diluted 1:5 (1 part sample:4 parts STR 1X Buffer) prior to loading. The bottom panel shows the relevant portion of the Fluorescent Ladder (CXR), 60–400 Bases. The numbers above the peaks indicate the sizes of the Fluorescent Ladder (CXR) fragments.

10. Representative STR Data (continued)

Artifacts and Stutter

Stutter bands are a common amplification artifact associated with STR analysis. Stutter products are often observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

In addition to stutter peaks, other artifact peaks can be observed. There is an artifact noted in the D5S818 typing region when amplifying the *GenePrint*® Fluorescent STR Multiplex—GammaSTR® System and using ABI PRISM® 3130 Genetic Analyzer for detection. This artifact has been characterized as secondary structure associated with the D16S539 primer set and migrates between 80 and 110bp, depending upon instrumentation. The artifact has broad peak morphology and should not fall on-ladder. In addition, samples are diluted as recommended in Section 7.B, 8.C or 9.B, the peak should fall below 300RFU.

11. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

Symptoms	Causes and Comments
Faint or no bands/allele peaks	Impure template DNA. Inhibitors may exist in the DNA sample.
	Insufficient template DNA. Use the recommended amount of template DNA.
	Insufficient enzyme activity. Use the recommended amount of *Taq* DNA polymerase. Check the expiration date on the tube label.
	Wrong amplification program. Choose the correct amplification program for each locus.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH may also affect PCR. Store DNA in TE^{-4} buffer (10mM Tris HCl [pH 8.0], 0.1mM EDTA) or nuclease-free water.
	Thermal cycler or tube problems. Review the thermal cycling protocols in Section 4. We have not tested other reaction tubes or thermal cyclers. Calibration of the thermal cycler heating block may be required.
	Primer concentration was too low. Use the recommended primer concentration. Mix well before use.
	Ice was not used to set up reactions. Set up the reactions on ice. Very light allele intensity is obtained with some loci if ice is not used when setting up the reactions. The use of AmpliTaq Gold® DNA polymerase will also remedy this problem.

Symptoms	Causes and Comments
Faint or no bands/allele peaks (continued)	Samples were not denatured before loading in the gel. Be sure the samples are heated at 95°C for 2 minutes and cooled on crushed ice or in an ice-water bath immediately prior to loading.
	Poor CE injection. Re-inject the sample.
	Poor-quality formamide. Be sure that high-quality formamide is used when running samples on the ABI PRISM® 310 or 3100 Genetic Analyzer.
Extra bands visible in one or all lanes	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Artifacts of STR amplification. PCR amplification sometimes generates artifacts that appear as faint bands one or four bases below an allele. Refer to Section 13.B for locus-specific information regarding this event.
	Samples were not completely denatured. Heat denature samples at 95°C for 2 minutes immediately prior to loading the gel.
	Insufficient pre-run of gel. Pre-run gels until a temperature of 50°C is reached before loading samples
Bands are fuzzy throughout the lanes	Poor-quality polyacrylamide gel. Prepare acrylamide and buffer solutions using high-quality reagents. Store acrylamide solutions in the dark.
	Electrophoresis temperature was too high. Run gel at a lower temperature (40–60°C).
Extra peaks visible in one or all color channels (ABI PRISM® 310 or 3100 Genetic Analyzer)	CE-related artifacts. Minor voltage changes or urea crystals passing by the laser may cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject the samples to confirm.
Allelic ladder not running the same as the sample	Wrong allelic ladder or primer pair mix was used. Be sure that the allelic ladder is from the same kit as the 10X Primer Pair Mix.
	Poor-quality formamide was used. Be sure that high-quality formamide is used when running samples on the ABI PRISM® 310 or 3100 Genetic Analyzer.
Uneven peak heights between loci	Thermal cycler problems. Review the thermal cycling protocols in Section 4. We have not tested other thermal cyclers.
	Excessive amount of DNA. Use the recommended amount of template. See Table 5, Section 4.A, for recommendations.
	Use of FTA® paper. Results may be similar to use of excess amounts of DNA template. Reduce the number of cycles in the amplification program by 2–4 cycles (10/18 or 10/16 cycling) to improve the locus-to-locus balance.
	Degraded DNA sample. DNA template is degraded into smaller fragments, with the larger loci showing diminished yield.
High background with low signal	Part of the spacers were scanned. Re-scan the gel being careful not to scan any portion of the spacers.
	Plates were improperly washed. Improper washing of the plates can cause a soap residue to build up on the plates. This can cause background fluorescence.

12. References

12.A. Cited References

1. Edwards, A. *et al.* (1991) DNA typing with trimeric and tetrameric tandem repeats: Polymorphic loci, detection systems, and population genetics. In: *Proceedings from The Second International Symposium on Human Identification* 1991, Promega Corporation, 31–52.
2. Edwards, A. *et al.* (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am. J. Hum. Genet.* **49**, 74–56.
3. Edwards, A. *et al.* (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* **12**, 241–53.
4. Warne, D. *et al.* (1991) Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene 2 (ACTBP2) detected using the polymerase chain reaction. *Nucleic Acids Res.* **19**, 6980.
5. Ausubel, F.M. *et al.* (1993) Unit 15: The polymerase chain reaction. In: *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, NY.
6. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Chapter 14: In vitro amplification of DNA by the polymerase chain reaction. In: *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
7. *PCR Technology: Principles and Applications for DNA Amplification* (1989) ed., Erlich, H.A., Stockton Press, NY.
8. *PCR Protocols: A Guide to Methods and Applications* (1990) eds., Innis, M.A. *et al.*, Academic Press, San Diego, CA.
9. Puers, C. *et al.* (1993) Identification of repeat sequence heterogeneity at the polymorphic STR locus HUMTH01[AATG]n and reassignment of alleles in population analysis using a locus-specific allelic ladder. *Am. J. Hum. Genet.* **53**, 953–8.
10. Hammond, H. *et al.* (1994) Evaluation of 13 short tandem repeat loci for use in personal identification applications. *Am. J. Hum. Genet.* **55**, 175–89.
11. Bever, R.A. and Creacy, S. (1995) Validation and utilization of commercially available STR multiplexes for parentage analysis. In: *Proceedings from the Fifth International Symposium on Human Identification* 1994, Promega Corporation, 61–8.
12. Sprecher, C.J. *et al.* (1996) General approach to analysis of polymorphic short tandem repeat loci. *BioTechniques* **20**, 266–76.
13. Lins, A.M. *et al.* (1996) Multiplex sets for the amplification of polymorphic short tandem repeat loci—silver stain and fluorescent detection. *BioTechniques* **20**, 882–9.
14. Presley, L.A. *et al.* (1992) The implementation of the polymerase chain reaction (PCR) HLA DQ alpha typing by the FBI laboratory. In: *Proceedings from the Third International Symposium on Human Identification* 1992, Promega Corporation, 245–69.
15. Hartmann, J.M. *et al.* (1991) Guidelines for a quality assurance program for DNA analysis. *Crime Laboratory Digest* **18**, 44–75.
16. Micka, K.A. *et al.* (1999) TWGDAM validation of a nine-locus and a four-locus fluorescent STR multiplex system. *J. Forensic Sci.* **44**, 1243–57.
17. Bassam, B.J., Caetano-Anolles, G. and Gresshoff, P.M. (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* **196**, 80–3.
18. Budowle, B. *et al.* (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. *Am. J. Hum. Genet.* **48**, 137–44.
19. Nakamura, Y. *et al.* (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* **235**, 1616–22.
20. Budowle, B. and Monson, K.L. (1989) In: *Proceedings of an International Symposium on the Forensic Aspects of DNA Analysis*, Government Printing Office, Washington, D.C.

21. Levinson, G. and Gutman, G.A. (1987) Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* **4**, 203–21.

22. Schlotterer, C. and Tautz, D. (1992) Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.* **20**, 211–5.

23. Smith, J.R. *et al.* (1995) Approach to genotyping errors caused by nontemplated nucleotide addition by *Taq* DNA polymerase. *Genome Res.* **5**, 312–7.

24. Magnuson, V.L. *et al.* (1996) Substrate nucleotide-determined non-templated addition of adenine by *Taq* DNA polymerase: Implications for PCR-based genotyping. *BioTechniques* **21**, 700–9.

25. Walsh, P.S., Fildes, N.J. and Reynolds, R. (1996) Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res.* **24**, 2807–12.

26. Puers, C. *et al.* (1994) Analysis of polymorphic STR loci using well-characterized allelic ladders. In: *Proceedings from the Fourth International Symposium on Human Identification* 1993, Promega Corporation, 161–72.

27. Puers, C. *et al.* (1994) Allelic ladder characterization of the short tandem repeat polymorphism located in the 5´ flanking region to the human coagulation factor XIII A subunit gene. *Genomics* **23**, 260–4.

28. Griffiths, R. *et al.* (1998) New reference allelic ladders to improve allelic designation in a multiplex STR system. *Int. J. Legal Med.* **111**, 267–72.

29. Bär, W. *et al.* (1997) DNA recommendations: Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int. J. Legal Med.* **110**, 175–6.

30. Gill, P. *et al.* (1997) Considerations from the European DNA Profiling Group (EDNAP) concerning STR nomenclature. *Forensic Sci. Int.* **87**, 185–92.

31. Bär, W. *et al.* (1997) DNA Recommendations: Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int. J. Leg. Med.* **110**, 175–6.

32. Mandrekar, P.V., Krenke, B.E. and Tereba, A. (2001) DNA IQ™: The intelligent way to purify DNA. *Profiles in DNA* **4**(3), 16.

33. Krenke, B.E. *et al.* (2005) Development of a novel, fluorescent, two-primer approach to quantitative PCR. *Profiles in DNA* **8(1)**, 3–5.

34. Greenspoon, S. and Ban, J. (2002) Robotic extraction of sexual assault samples using the Biomek® 2000 and the DNA IQ™ System. *Profiles in DNA* **5(1)**, 3–5.

35. McLaren, B., Bjerke, M. and Tereba, A. (2006) Automating the DNA IQ™ System on the Biomek® 3000 Laboratory Automation Workstation. *Profiles in DNA* **9(1)**, 11–3.

36. Cowan, C. (2006) The DNA IQ™ System on the Tecan Freedom EVO® 100. *Profiles in DNA* **9(1),** 8–10.

37. Bjerke, M. *et al.* (2006) Forensic application of the Maxwell™ 16 Instrument. *Profiles in DNA* **9(1)**, 3–5.

38. Mandrekar, P. *et al.* (2007) Introduction to Maxwell® 16 low elution volume configuration for forensic casework. *Profiles in DNA* **10(2),** 10–2.

39. Grimberg, J. *et al.* (1989) A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res.* **17**, 8390.

40. Miller, S., Dykes, D. and Polesky, H. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.

41. Comey, C. *et al.* (1994) DNA extraction strategies for amplified fragment length polymorphism analysis. *J. Forensic Sci.* **39**, 1254–69.

42. Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**, 506–13.

43. Higuchi, R. (1989) Rapid, efficient DNA extraction for PCR from cells or blood. In: *Amplifications: A Forum for PCR Users* (May 1989) PerkinElmer, Norwalk, CT, Issue 2.

12.B. Additional STR References

A substantial reference list of publications describing STRs and related information can be found at a web site created by the National Institutes of Standards and Technology (NIST) Biotechnology Division. This web site (**www.cstl.nist.gov/div831/strbase/**) is occasionally updated and has numerous links to many other useful sites.

The references below provide an overview of the listed topics and are available at: **www.promega.com/profiles/**. The spreadsheet for the PowerStats application is available at: **www.promega.com/geneticidtools/powerstats/**. PowerStats is a Microsoft® Excel workbook spreadsheet that allows genotype data from STaRCall™ or Genotyper® software to be pasted directly into the workbook to obtain standard population statistics on the distribution of alleles within particular population subsets. If you cannot access the web site, please contact your local Promega branch office or distributor.

1. **The Short Tandem Repeat DNA Database Web Site:**

   Butler, J.M. and Reeder, D.J. (1997) STRBase: A short tandem repeat DNA database. *Profiles in DNA* **1(2)**, 10.

2. **PowerStats Analysis of Population Data:**

   Tereba, A. (1999) Tools for analysis of population statistics. *Profiles in DNA* **2(3)**, 14–6.

13. Appendix

13.A. Advantages of STR Typing

The *GenePrint*® Fluorescent STR Systems provide a rapid, non-radioactive method to evaluate small amounts (e.g., 1ng) of human DNA. The protocols in this manual describe the use of a fluorescein label to detect amplified STR products following their separation by denaturing polyacrylamide gel electrophoresis. For information on multicolor fluorescent STR systems, refer to the *PowerPlex® 16 System Technical Manual* #TMD012, *PowerPlex® 16 BIO System Technical Manual* #TMD016, *PowerPlex® ES System Technical Manual* #TMD017, *PowerPlex® 1.1 System Technical Manual* #TMD008, *PowerPlex® 1.2 System Technical Manual* #TMD009 or *PowerPlex® 2.1 System Technical Manual* #TMD011. Refer to the *GenePrint® STR Systems Technical Manual* #TMD004 for information about detecting STR products using silver staining (17). These Technical Manuals are available at: **www.promega.com/tbs/**

STR typing is more tolerant of degraded DNA templates than other methods of individual identification because amplification products are less than 400bp long, much smaller than the material detected with AMP-FLP (18) or VNTR (19) analysis. This format is also amenable to a variety of rapid DNA purification techniques.

In addition to these advantages, the STR loci chosen for inclusion in the *GenePrint*® systems contain alleles of discrete and separable lengths. This allows the construction of allelic ladders, which contain fragments of the same lengths as several or all known alleles for the locus. Visual comparison between the allelic ladder and amplified samples of the same locus allows rapid and precise assignment of alleles. Results obtained using the *GenePrint*® Fluorescent STR Systems can be recorded in a digitized format, allowing direct comparison with stored databases. Population analyses do not require the use of arbitrarily defined fixed bins for population data (20).

13.B. Advantages of Using the Loci in the *GenePrint*® Fluorescent STR Systems

The STR loci and primers contained in the *GenePrint*® Fluorescent STR Systems (Tables 12 and 13) have been carefully selected to minimize artifacts, including those associated with *Taq* DNA polymerase such as repeat slippage and terminal nucleotide addition, as well as genetic artifacts called microvariant alleles. Repeat slippage (21,22), sometimes called "n–4 bands," "stutter" or "shadow bands", is due to the loss of a repeat unit during DNA amplification. The amount of this artifact observed depends primarily on the locus and the DNA sequence being replicated. We have chosen loci that exhibit little or no repeat slippage. The vWA locus is an exception, revealing as much as 10% stutter. This locus has been included primarily for its popularity in the forensics community.

Terminal nucleotide addition (23,24) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact band one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. Redefinition of the primer sequences and/or addition of a final extension step of 60°C for 30 minutes to the amplification protocol can lead to essentially full terminal nucleotide addition (25).

Notes for Table 13:

1. PCR amplification sometimes generates artifacts that appear as faint bands below the alleles. These products probably result from a process known as slippage, commonly observed in PCR amplification of regions containing tandem repeats of short sequences (21,22). This characteristic is most pronounced with the vWA locus.
2. A strong extra band may be observed below the 212bp Amelogenin allele when more than 25ng of template DNA is amplified.
3. Locus F13A01 has a common allele 3.2. It contains four copies of the repeat but has a two-base deletion in the region flanking the repeat (26,27).
4. Locus TH01 has a common 9.3 allele (9). A one-base deletion is present in the allele that contains 10 repeats. Note that reference 9 refers to this allele as 10–1 rather than 9.3. This allele was renamed 9.3 at the ISFH Conference in Venice in October 1993.

Table 12. Locus-Specific Information.

STR Locus	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence[2] 5´→3´
Amelogenin[1]	Xp22.1–22.3 and Y	HUMAMEL, Human Y chromosomal gene for amelogenin-like protein	NA
CSF1PO	5q33.3–34	HUMCSF1PO, Human c-fms proto-oncogene for CSF-1 receptor gene	AGAT
D16S539	16q24–qter	NA	AGAT
D7S820	7q11.21–q22	NA	AGAT
D13S317	13q22–q31	NA	AGAT
D5S818	5q23.3–32	NA	AGAT
F13A01	6p24.3–p25.1	HUMF13A01, Human coagulation factor XIII a subunit gene	AAAG
F13B	1q31–q32.1	HUMBFXIII, Human factor XIII b subunit gene	AAAT
FESFPS	15q25–qter	HUMFESFPS, Human c-fes/fps proto-oncogene	AAAT
HPRTB	Xq26	HUMHPRTB, Human hypoxanthine phosphoribosyl-transferase gene	AGAT
LPL	8p22	HUMLIPOL, Human lipoprotein lipase gene	AAAT
TH01	11p15.5	HUMTH01, Human tyrosine hydroxylase gene	AATG
TPOX	2p24–pter	HUMTPOX, Human thyroid peroxidase gene	AATG
vWA	12p13.31	HUMVWFA31, Human von Willebrand factor gene	TCTA Complex (28)

NA = not applicable.

[1]Amelogenin is not an STR, but displays a 212-base, X-specific band and a 218-base, Y-specific band. K562 DNA (female) displays only the 212-base, X-specific band.

[2]The August 1997 report (29,30) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

Table 13. Additional Locus-Specific Information.

STR Locus	Allelic Ladder Size Range[1] (bases)	STR Ladder Alleles (# of repeats)	Other Known Alleles[2] (# of repeats)	K562 DNA Allele Sizes (# of repeats)	Comments
Amelogenin[3]	212–218	NA	None	212,212	1,2
CSF1PO	295–327	7,8,9,10,11, 12,13,14,15	6	10,9	1
D16S539	264–304	5,8,9,10,11, 12,13,14,15	None	12,11	1
D7S820	215–247	6,7,8,9,10, 11,12,13,14	None	11,9	1
D13S317	165–197	7,8,9,10,11, 12,13,14,15	None	8,8	1
D5S818	119–151	7,8,9,10,11, 12,13,14,15	16	12,11	1
F13A01	283–331	4,5,6,7,8,9,11, 12,13,14,15,16	3.2,10[4]	5,4[5]	1,3
F13B	169–189	6,7,8,9,10,11	12	10,10	1
FESFPS	222–250	7,8,9,10,11,12,13,14	None	12,10	1
HPRTB	259–303	6,7,**8**,9,10,**11**, 12,13,**14**,15,16,**17**	None	13,13	1
LPL	105–133	7,9,10,11,12,13,14	8	12,10	1
TH01	179–203	5,6,7,8,9,10,11	9.3	9.3,9.3	1,4
TPOX	224–252	6,7,8,9,10,11,12,13	None	9,8	1
vWA	139–167	13,14,15,16, 17,18,19,20	10,11, 21,22	16,16	1

NA = not applicable.

Alleles in bold are present in greater amounts than other alleles. This simplifies interpretation.

[1]Lengths of each allele in the allelic ladders have been confirmed by sequence analyses.

[2]Alleles that represent <0.2% of the population may not be listed in this table.

[3]Amelogenin is not an STR, but displays a 212 base, X-specific band and a 218 base, Y-specific band. K562 DNA (female) displays only the 212 base X-specific band.

[4]Allele 10 (307 bases) is not included because it is rare and its exclusion creates a gap that simplifies interpretation of the allelic ladder (27,31).

[5]F13A01 allele 5 appears more intense than allele 4 in the K562 control sample. The K562 strain contains an unusual number of chromosomes, and some are represented more than twice per cell. It is hypothesized that in this strain the allele 5 version of chromosome 6 is present twice, while the allele 4 version of chromosome 6 is present only once.

13.C. Power of Discrimination

The *GenePrint*® Fluorescent STR Systems provide extremely powerful discrimination. The combined matching probability of the CTTv, FFFL and GammaSTR® quadriplexes range from 1 in 303,000,000,000 in Caucasian-Americans to 1 in 4,610,000,000,000 in African-Americans (Table 14).

Typical paternity indices for the *GenePrint*® Fluorescent STR Systems are shown in Table 15. An alternative calculation in paternity analyses is the power of exclusion. Table 16 gives typical values for the power of exclusion for the *GenePrint*® Fluorescent STR Systems in various populations.

Table 14. Matching Probability of Various Populations.

	Matching Probability		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
CTTv quadriplex (CSF1PO, TPOX, TH01, vWA)	1 in 25,236	1 in 6,796	1 in 7,219
FFFL quadriplex (F13A01, FESFPS, F13B, LPL)	1 in 16,802	1 in 2,658	1 in 3,276
GammaSTR® III quadriplex (D16S539, D7S820, D13S317, D5S818)	1 in 10,872	1 in 16,790	1 in 20,106
All 3 quadriplexes (12 loci)	1 in 4.61×10^{12}	1 in 3.03×10^{11}	1 in 4.75×10^{11}

Table 15. Typical Paternity Indices of the Multiplex *GenePrint*® STR Systems in Various Populations.

	Typical Paternity Index		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
CTTv quadriplex (CSF1PO, TPOX, TH01, vWA)	29.4	19.26	10.51
FFFL quadriplex (F13A01, FESFPS, F13B, LPL)	16.83	15.28	8.23
GammaSTR® III quadriplex (D16S539, D7S820, D13S317, D5S818)	16.93	13.51	30.40
All 3 quadriplexes (12 loci)	8,373	3,976	2,627

Table 16. Power of Exclusion of the *GenePrint*® STR Systems in Various Populations.

	Power of Exclusion		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
CTTv quadriplex (CSF1PO, TPOX, TH01, vWA)	0.967	0.953	0.918
FFFL quadriplex (F13A01, FESFPS, F13B, LPL)	0.946	0.941	0.902
GammaSTR® III quadriplex (D16S539, D7S820, D13S317, D5S818)	0.946	0.934	0.967
All 3 quadriplexes (12 loci)	0.99990	0.99981	0.99974

13.D. DNA Extraction and Quantitation Methods

The DNA IQ™ System (Cat.# DC6700) is a DNA isolation and quantitation system designed specifically for forensic and paternity samples (32). This novel technology uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. For larger samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate and quantify DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. See Section 13.G for additional information.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1000) has been developed (33). See Section 13.G for ordering information.

The DNA IQ™ System has been fully automated on the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation (34), Biomek® 3000 Laboratory Automation Workstation (35) and Tecan Freedom EVO® Liquid Handler (36). In addition, the DNA IQ™ Reference Sample Kit for Maxwell® 16 (Cat.# AS1040) and DNA IQ™ Casework Sample Kit for Maxwell® 16 (Cat.# AS1210) are available (37,38). For information on automation of laboratory processes on automated workstations, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com/worldwide/**) or e-mail: **genetic@promega.com**

Traditional DNA extraction methods, such as phenol:chloroform extraction methods (32) or inorganic methods (39,40), can be used for STR analysis. For stains from blood and saliva, scientists at the FBI Academy have suggested an alternative method for DNA extraction (see reference 41). DNA isolation methods such as Chelex® 100 (42) and cell lysis followed by proteinase K digestion (43) are capable of extracting DNA from bloodstains or from as little as 3µl of whole blood. Because both of these methods produce single-stranded DNA, they should not be used for VNTR (variable number tandem repeat) polymorphism analysis.

13.E. Agarose Gel Electrophoresis of Amplification Products (Optional)

This procedure is optional if PCR is routinely performed in your laboratory. Agarose gel electrophoresis can be used to rapidly confirm the success of the amplification reaction prior to performing polyacrylamide gel.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- TAE 1X buffer
- agarose
- 5X loading solution
- ethidium bromide solution, 0.5µg/ml

Ethidium bromide is a powerful mutagen. Wear gloves at all times, and use a mask when weighing out ethidium bromide powder.

1. Prepare a 2% agarose gel (approximately 150cm^2) by adding 2.0g of agarose to 100ml of TAE 1X buffer. Mark the liquid level on the container, then boil or heat in a microwave oven to dissolve the agarose. Add preheated (60°C) deionized water to make up for any volume lost due to evaporation.
2. Cool the agarose to 55°C before pouring into the gel tray. Be sure that the gel tray is level. Pour the agarose into the tray, insert the gel comb and allow to set for 20–30 minutes.
3. Prepare samples by mixing 10µl of each amplified sample with 2.5µl of 5X loading solution.
4. Prepare 1 liter of TAE 1X buffer for the electrophoresis running buffer.
5. Place the gel and tray in the electrophoresis gel box. Pour enough running buffer into the tank to cover the gel to a depth of at least 0.65cm. Gently remove the comb.
6. Load each sample mixed with 5X loading solution (prepared in Step 3).
7. Set the voltage at 5 volts/cm (measured as the distance between the two electrodes). Allow the gel to run for 2 hours.
8. After electrophoresis, stain the gel in TAE 1X buffer containing 0.5µg/ml ethidium bromide. Gently rock for 20 minutes at room temperature. Remove the ethidium bromide solution, and replace with deionized water. Allow the gel to destain for 20 minutes.
9. Using a UV transilluminator (302nm), photograph the gel (e.g., with Polaroid® 667 film).

   Note: When analyzing data, do not be alarmed by extra bands in addition to the alleles. DNA heteroduplexes can be expected when performing nondenaturing agarose gel electrophoresis. The sole purpose of the agarose gel is to confirm PCR success.

13.F. Composition of Buffers and Solutions

40% acrylamide:bis (19:1)

Dissolve 380g of acrylamide and 20g of bisacrylamide in 500ml of deionized water. Bring volume to 1 liter with deionized water.

10% ammonium persulfate

Add 0.5g of ammonium persulfate to 5ml of deionized water. Use 200µl for 30ml of acrylamide gel solution. Store the remaining volume in 200µl aliquots at –20°C.

Blue Dextran Loading Solution

4.1mM	EDTA
88.25%	formamide, ACS grade
15mg/ml	blue dextran

Bromophenol Blue Loading Solution

10mM	NaOH
95%	formamide
0.05%	bromophenol blue

0.5M EDTA (pH 8.0)

186.1g	$Na_2EDTA \cdot 2H_2O$

Add EDTA to 800ml of deionized water with vigorous stirring. Adjust the pH to 8.0 with NaOH (about 20g of NaOH pellets). Adjust final volume to 1 liter. Dispense into aliquots, and sterilize by autoclaving.

ethidium bromide solution (10mg/ml)

1.0g	ethidium bromide

Dissolve in 100ml of deionized water. Wrap in aluminum foil or transfer to a dark bottle, and store at room temperature.

Caution: Ethidium bromide is a powerful mutagen. Wear gloves at all times, and use a mask when weighing out ethidium bromide powder.

Gel Tracking Dye

10mM	NaOH
95%	formamide
0.05%	bromophenol blue
0.05%	xylene cyanol FF

Gold ST*R 10X Buffer

500mM	KCl
100mM	Tris-HCl (pH 8.3 at 25°C)
15mM	$MgCl_2$
1%	Triton® X-100
2mM	each dNTP
1.6mg/ml	BSA

5X loading solution

5%	Ficoll® 400
0.1%	bromophenol blue
0.1%	xylene cyanol
100mM	EDTA ($Na_2EDTA \cdot 2H_2O$)
10mM	Tris-HCl (pH 7.5)

STR 2X Loading Solution

10mM	NaOH
95%	formamide
0.05%	bromophenol blue
0.05%	xylene cyanol FF

STR 10X Buffer

500mM	KCl
100mM	Tris-HCl (pH 9.0)
15mM	$MgCl_2$
1%	Triton® X-100
2mM	each dNTP

50X TAE buffer (pH 7.2)

242g	Tris base
57.1ml	glacial acetic acid
100ml	0.5M EDTA (pH 8.0)

Add Tris base and EDTA to 500ml of deionized water. Add the glacial acetic acid. Bring the volume to 1 liter with deionized water.

13.F. Composition of Buffers and Solutions (continued)

1X TAE buffer (pH 7.2)

Add 20ml of 50X TAE buffer to 980ml of deionized water.

10X TBE buffer

107.8g	Tris base
7.44g	EDTA ($Na_2EDTA \cdot 2H_2O$)
~55.0g	boric acid

Dissolve Tris base and EDTA in 800ml of deionized water. Slowly add the boric acid, and monitor the pH until the desired pH of 8.3 is obtained. Bring the volume to 1 liter with deionized water.

0.5X TBE buffer

Add 50ml of 10X TBE buffer to 950ml of deionized water.

13.G. Related Products

Fluorescent STR Multiplex Systems

Product	Size	Cat.#
PowerPlex® 1.1 System	100 reactions	DC6091
	400 reactions	DC6090
PowerPlex® 2.1 System	100 reactions	DC6471
	400 reactions	DC6470
PowerPlex® 1.2 System	100 reactions	DC6101
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® 16 BIO System	100 reactions	DC6541
	400 reactions	DC6540
PowerPlex® ES System	100 reactions	DC6731
	400 reactions	DC6730

Not for Medical Diagnostic Use.

The PowerPlex® 1.1, 2.1, and 16 BIO Systems are compatible with the Hitachi FMBIO® Fluorescence Imaging Systems.

Accessory Components

Product	Size	Cat.#
Acrylamide	100g	V3111
Ammonium Persulfate	25g	V3131
Bisacrylamide	25g	V3141
Blue Dextran Loading Solution	3ml	DV4351
Bromophenol Blue Loading Solution	3ml	DV4371
Gel Tracking Dye	1ml	DV4361
Gold ST*R 10X Buffer	1.2ml	DM2411
K562 DNA High Molecular Weight	30µg	DD2011
Mineral Oil	12ml	DY1151
Nuclease-Free Water	50ml	P1193
	150ml	P1195
PowerPlex® Matrix Standards, 310**	50µl (each dye)	DG4640
PowerPlex® Matrix Standards, 3100/3130**	25µl (each dye)	DG4650
STR 10X Buffer	1.2ml	DM2211
STR 2X Loading Solution	3ml	DV4331
TBE Buffer, 10X	1L	V4251
Urea	1kg	V3171

**Not for Medical Diagnostic Use.

Internal Lane Standards

Product	Size	Cat.#
Internal Lane Standard 600	150µl	DG1071
Fluorescent Ladder CXR, 60–400 Bases	65µl	DG6221

Sample Preparation Systems

Product	Size	Cat.#
Plexor® HY System*	200 reactions	DC1001
	800 reactions	DC1000
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
DNA IQ™ Casework Sample Kit for Maxwell® 16	48 preps	AS1210
DNA IQ™ Reference Sample Kit for Maxwell® 16	48 preps	AS1040
Differex™ System*	50 samples	DC6801
	200 samples	DC6800
Slicprep™ 96 Device	10 pack	V1391

*Not for Medical Diagnostic Use.

ART® Aerosol-Resistant Tips

Product	Volume	Size (tips/pack)	Cat.#
ART® 10 Ultramicro Pipet Tip	0.5–10µl	960	DY1051
ART® 20E Ultramicro Pipet Tip	0.5–20µl	960	DY1061
ART® 20P Pipet Tip	20µl	960	DY1071
ART® GEL Gel Loading Pipet Tip	100µl	960	DY1081
ART® 100 Pipet Tip	100µl	960	DY1101
ART® 100E Pipet Tip	100µl	960	DY1111
ART® 200 Pipet Tip	200µl	960	DY1121
ART® 1000E Pipet Tip	1,000µl	800	DY1131

[a]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759, Australian Pat. No. 670231 and other pending patents assigned to Baylor College of Medicine, Houston, Texas.

Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

[b]U.S. Pat. Nos. 5,843,660 and 6,221,598, Australian Pat. No. 724531, Canadian Pat. No. 2,118,048, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. No. 3602142 and other patents pending.

[c]U.S. Pat. No. 5,843,660, Australian Pat. No. 724531, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. No. 3602142 and other patents pending.

[d]U.S. Pat. No. 6,221,598, Canadian Pat. No. 2,118,048, Korean Pat. No. 290332 and other patents pending.

© 2012 Promega Corporation. All Rights Reserved.

GammaSTR, *GenePrint*, Maxwell, Plexor and PowerPlex are registered trademarks of Promega Corporation. Differex, DNA IQ and Slicprep are trademarks of Promega Corporation.

ABI PRISM, GeneScan, Genotyper and MicroAmp are registered trademarks of Applera Corporation. AmpliTaq, AmpliTaq Gold and GeneAmp are registered trademarks of Roche Molecular Systems, Inc. ART is a registered trademark of Molecular BioProducts, Inc. Biomek is a registered trademark of Beckman Coulter, Inc. Chelex is a registered trademark of Bio-Rad Laboratories, Inc. Ficoll is a registered trademark of GE Healthcare Bio-sciences. FMBIO is a registered trademark of Hitachi Software Engineering Company, Ltd. Freedom EVO is a registered trademark of Tecan AG Corporation. FTA is a registered trademark of Flinders Technologies, Pty, Ltd., and is licensed to Whatman. GenBank is a registered trademark of the U.S. Dept. of Health and Human Services. Hi-Di and POP-4 are trademarks of Applera Corporation. Kimwipes is a registered trademark of Kimberly-Clark. Liqui-Nox is a registered trademark of Alconox, Inc. Long Ranger is a registered trademark of Cambrex Corporation. Microsoft is a registered trademark of Microsoft Corporation. Nalgene is a registered trademark of Nalge Nunc International. Polaroid is a registered trademark of Polaroid Corporation. STaRCall is a trademark of Hitachi Software Engineering Company, Ltd. Triton is a registered trademark of Union Carbide Chemicals and Plastics Technology Corporation.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.